**Devices and Methods for Using Centripetal Acceleration to Drive Fluid Movement
in a Microfluidics System for Performing Biological Fluid Assays**

This application claim priority to U.S. Serial No. 09/083,678, filed May 22, 1998. This application is also related to U.S. Serial No. 08/995,056, filed December 19, 1997, U.S. Serial No. 08/910,726, filed August 12, 1997, U.S. Serial No. 08/768,990, filed December 18, 1996 and U.S. Serial No. 08/761,063, filed December 5, 1996, the disclosures of every one of which are explicitly incorporated by reference herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to methods and apparatus for performing microanalytic analyses and procedures on fluid samples. In particular, the invention relates to microminiaturization of genetic, biochemical and chemical processes related to analysis, synthesis and purification. Specifically, the invention provides a microsystem platform that is rotationally manipulated by a micromanipulation device, thereby utilizing the centripetal forces resulting from rotation of the platform to motivate fluid movement through microchannels embedded in the microplatform. The microsystem platforms of the invention are provided having microfluidics components, chambers and reservoirs, resistive heating elements, temperature sensing elements, mixing structures, capillary and sacrificial valves, and methods for using these microsystems platforms for performing biological, enzymatic, immunological and chemical assays.

2. Summary of the Related Art

Assays for detecting analytes in fluid samples, particularly complex fluids such as biological fluid samples, are used for a variety of diagnostic, environmental, synthetic and analytical purposes in the medical, biological, chemical, biochemical and environmental arts.

Certain analytes are important for diagnosis and monitoring of acute and chronic disease in humans. For example, diabetes is the fourth major cause of morbidity and mortality in the U.S., even though the biochemical basis for the disease has been known for at least a century and drugs (primarily insulin) and methods for managing the disease are robust and widely used. It has been recognized that sensitive monitoring of blood sugar levels, either directly or by detecting levels of metabolites and disease-associated modifications (such as the relative fraction of glycated hemoglobin in the bloodstream) is important in managing the disease. However, fast and

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inexpensive ways of performing the assays necessary for efficient management of multiple indicators of the disease state are not currently available.

In the field of medical, biological and chemical assays, mechanical and automated fluid handling systems and instruments are known in the prior art.

U.S. Patent No. 4,279,862, issued July 21, 1981 to Bertaudiere *et al.* disclose a centrifugal photometric analyzer.

U.S. Patent No. 4,381,291, issued April 26, 1983 to Ekins teach analytic measurement of free ligands.

U.S. Patent No. 4,515,889, issued May 7, 1985 to Klose *et al.* teach automated mixing and incubating reagents to perform analytical determinations.

U.S. Patent No. 4,676,952, issued June 30, 1987 to Edelmann *et al.* teach a photometric analysis apparatus.

U.S. Patent No. 4,745,072, issued May 17, 1998 to Ekins discloses immunoassay in biological fluids.

U.S. Patent No. 5,061,381, issued October 29, 1991 to Burd discloses a centrifugal rotor for performing blood analyses.

U.S. Patent No. 5,122,284, issued June 16, 1992 to Braynin *et al.* discloses a centrifugal rotor comprising a plurality of peripheral cuvettes.

U.S. Patent No. 5,160,702, issued November 3, 1993 to Kopf-Sill and Zuk discloses rotational frequency-dependent ❑valves❑ using capillary forces and siphons, dependent on ❑wettablility" of liquids used to prime said siphon.

U.S. Patent No. 5,171,695, issued December 15, 1992 to Ekins discloses determination of analyte concentration using two labeling markers.

U.S. Patent No. 5,173,193, issued December 22, 1992 to Schembri discloses a centrifugal rotor for delivering a metered amount of a fluid to a receiving chamber on the rotor.

U.S. Patent No. 5,242,803, issued September 7,1993 to Burtis *et al.* disclose a rotor assembly for carrying out an assay.

U.S. Patent No. 5,409,665, issued April 25, 1995 to Burd discloses a cuvette filling in a centrifuge rotor.

U.S. Patent No. 5,413,009, issued July 11, 1995 to Ekins discloses a method for analyzing analytes in a liquid.

U.S. Patent No. 5,472,603, issued December 5, 1995 to Schembri discloses an analytical rotor comprising a capillary passage having an exit duct wherein capillary forces prevent fluid flow

5 at a given rotational speed and permit flow at a higher rotational speed.

Anderson, 1968, *Anal. Biochem.* 28: 545-562 teach a multiple cuvette rotor for cell fractionation.

Renoe et al., 1974 *Clin. Chem.* 20: 955-960 teach a □minidisc□ module for a centrifugal analyzer.

10 Burtis et al., 1975, *Clin. Chem.* 20: 932-941 teach a method for a dynamic introduction of liquids into a centrifugal analyzer.

Fritsche et al., 1975, *Clin. Biochem.* 8: 240-246 teach enzymatic analysis of blood sugar levels using a centrifugal analyzer.

Burtis et al., 1975, *Clin Chem.* 21: 1225-1233 teach a multipurpose optical system for use

15 with a centrifugal analyzer.

Hadjiioannou et al., 1976, *Clin. Chem.* 22: 802-805 teach automated enzymatic ethanol determination in biological fluids using a miniature centrifugal analyzer.

Lee et al., 1978, *Clin. Chem.* 24: 1361-1365 teach a automated blood fractionation system.

Cho et al., 1982, *Clin. Chem.* 28: 1956-1961 teach a multichannel electrochemical

20 centrifugal analyzer.

Bertrand et al., 1982, *Clinica Chimica Acta* 119: 275-284 teach automated determination of serum 5'-nucleotidase using a centrifugal analyzer.

Schembri et al., 1992, *Clin Chem.* 38: 1665-1670 teach a portable whole blood analyzer.

Walters et al., 1995, Basic Medical Laboratory Technologies, 3rd ed., Delmar Publishers:

25 Boston teach a variety of automated medical laboratory analytic techniques.

Recently, microanalytical devices for performing select reaction pathways have been developed.

U.S. Patent No. 5,006,749, issued April 9, 1991 to White disclose methods apparatus for using ultrasonic energy to move microminiature elements.

U.S. Patent No. 5,252,294, issued October 12, 1993 to Kroy *et al.* teach a micromechanical structure for performing certain chemical microanalyses.

U.S. Patent 5,304,487, issued April 19, 1994 to Wilding *et al.* teach fluid handling on microscale analytical devices.

5   U.S. Patent 5,368,704, issued November 29, 1994 to Madou *et al.* teach microelectrochemical valves.

International Application, Publication No. WO93/22053, published 11 November 1993 to University of Pennsylvania disclose microfabricated detection structures.

International Application, Publication No. WO93/22058, published 11 November 1993 to
10   University of Pennsylvania disclose microfabricated structures for performing polynucleotide amplification.

Columbus *et al.*, 1987, *Clin. Chem.* <u>33</u>: 1531-1537 teach fluid management of biological fluids.

Ekins *et al.*, 1994 *Ann. Biol. Clin.* <u>50</u>: 337-353 teach a multianalytic microspot
15   immunoassay.

Wilding *et al.*, 1994, *Clin. Chem.* <u>40</u>: 43-47 disclose manipulation of fluids on straight channels micromachined into silicon.

One drawback in the prior art microanalytical methods and apparati has been the difficulty in designing systems for moving fluids on microchips through channels and reservoirs having
20   diameters in the 10-500μm range. Microfluidic systems require precise and accurate control of fluid flow and valving to control chemical reactions and analyte detection. Conventional pumping and valving mechanisms have been difficult to incorporate into microscale structures due to inherent conflicts-of-scale. These conflicts of scale arise in part due to the fact that molecular interactions arising out of mechanical components of such components, which are negligible in large
25   (macroscopic) scale devices, become very significant for devices built on a microscopic scale.

While devices and pumping and valving mechanisms have been developed which overcome some of these conflict-of-scale difficulties, there are other inherent problems with these systems. A number of microanalytical platforms have been developed which use electrokinetic forces for fluid pumping: electroosmotic flow devices; electrohydrodynamic devices; and electrophoretic

devices. An inherent drawback in these systems is that they rely on precise control of pH and free

charges in the fluid being pumped. This makes them incapable of pumping most raw biological

samples, such as blood and urine, and creates difficulties in pumping organic solvents. In cases

where these systems may be used, pre-conditioning of the fluid to enhance electrokinetic effects is

5   usually required.

Systems that use centripetal force to effect fluid movement in microstructures address the

need for a pumping mechanism to effect fluid flow, but cannot alone solve these scale-related

drawbacks of conventional fluidics reduced to microfluidics scale. There remains a need for a

simple, flexible, reliable, rapid and economical microanalytic and microsynthetic reaction platform

10   for performing biological, biochemical and chemical analyses and syntheses that can move fluids

within the structural components of a microsystems platform. Such a platform should be able to

move nanoliter-to microliter amounts of fluid, including reagents and reactants, at rapid rates to

effect the proper mixing of reaction components, removal of reaction side products, and isolation

of desired reaction products and intermediates. There remains a need in the art for centripetally-

15   motivated microfluidics platforms capable of precise and accurate control of flow and metering of

fluids in both microchip-based and centrifugal microplatform-based technologies.

SUMMARY OF THE INVENTION

20   This invention provides microsystems platforms as disclosed in co-owned and co-pending

U.S. Serial No. 08/761,063, filed December 5, 1996 and U.S. Serial No. 09/083,678, filed May 22,

1998, each of which is incorporated by reference herein. Specifically, this invention provides

microfluidics platforms for performing biological, enzymatic, immunological and chemical assays.

It is an advantage of the centrifugal rotors and microsystems platforms of the invention that

25   an imprecise amount of a fluid comprising a biological sample can be applied to the rotor or platform

and a precise volumetric amount of the biological sample is delivered to a fluid reservoir comprising

a reaction vessel or other component of the rotor or platform for performing chemical, biochemical,

immunological or other analyses. It is an advantage of the centrifugal rotors and microsystems

platforms of the invention that metering of said precise amount of a biological fluid sample, for

example, a drop of blood, is provided as an intrinsic property of the metering capillary channel of the rotor or platform, thereby avoiding variability introduced by centripetal metering of the sample into a reaction reservoir. It is a further advantage of the centrifugal rotors and microsystems platforms of the invention that an operator can avoid having to precisely measure an amount of a

5   fluid comprising a biological sample for application to the rotor or microsystem platform, thereby permitting end-users, including consumers, having a lower level of sophistication to use a medically diagnostic or other embodiment of the rotor or microsystem platform of the invention.

It is an advantage of the centrifugal rotors and microsystems platforms of the invention that fluid movement into and out of fluid reservoirs on the rotor or platform is precisely determined by

10   displacement of a first fluid, such as biological sample, from a fluid reservoir by a second fluid contained in a second reservoir on the rotor or platform. It is also an advantage of the centrifugal rotors and microsystems platforms of the invention that approximately complete replacement of the volumetric capacity of a first reservoir can be achieved by using fluid displacement as disclosed herein, thereby providing for maximum recovery of a first fluid sample upon displacement by a

15   second fluid, or maximum delivery and replacement of the first fluid by the second fluid. This aspect of the invention is advantageous for providing sequential chemical or biochemical reaction steps wherein mixing of the reagents is not desired.

It is also an advantage of the centrifugal rotors and microsystems platforms of the invention that these platforms provide an integrated microfluidics system containing components and

20   structures for performing microanalytic assays whereby fluid flow on the platform is motivated by centripetal force and controlled by capillary and/or sacrificial valves. The invention provides such integrated platforms whereby an operator is required simply to apply a sample, most preferably an imprecise volume of a fluid sample, to an entry port on the disk surface, and a complex series of analytical steps are performed without further operator manipulation on the platform. Movement

25   of fluids on the disk, and the sequence of analytical reaction steps performed thereupon, is a consequence of changes in rotor speed and/or the opening of sacrificial valves as directed by an instruction set contained in a program contained on the disk itself or in the memory of the micromanipulation apparatus that controls disk rotation and performance.

The microsystems platforms also provide disks that can perform a multiplicity of analytical

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reactions on either several samples or a particular sample, whereby the reactions are performed sequentially or individually. In addition, a wide variety of analytic reactions can be performed on the microsystems platforms of the invention, as further described below.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

Figures 1 through 8 are schematic representations of microfluidics arrays and components for performing direct analyte detection assays using the microsystems platforms of the invention.

Figures 9 through 11 are schematic representations of microfluidics arrays and components for performing separations of analyte from a fluid sample using the microsystems platforms of the invention.

Figures 12 and 13 are schematic representations of microfluidics arrays and components for performing both direct analyte detection assays and analyte separations using the microsystems platforms of the invention.

Figures 14 A and 14B illustrate the results of the assays disclosed in Example 4

Figure 15 is a photograph showing DNA recovery using the assays described in Example 4.

Figure 16 is a schematic diagram of a snap-in glucose assay component of a microsystems platform of the invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides centrifugal rotors and microsystems platforms for providing centripetally-motivated fluid micromanipulation.

For the purposes of this invention, the term "sample" will be understood to encompass any fluid, solution or mixture, either isolated or detected as a constituent of a more complex mixture, or synthesized from precursor species.

For the purposes of this invention, the term "in fluid communication" or "fluidly connected" is intended to define components that are operably interconnected to allow fluid flow

between components. In preferred embodiments, the platform comprises a rotatable platform, more preferably a disk, whereby fluid movement on the disk is motivated by centripetal force upon rotation of the disk.

For the purposes of this invention, the term "a centripetally motivated fluid micromanipulation apparatus" is intended to include analytical centrifuges and rotors, microscale centrifugal separation apparati, and most particularly the microsystems platforms and disk handling apparati of International Application No. WO97/21090, incorporated by reference.

For the purposes of this invention, the term "microsystems platform" is intended to include centripetally-motivated microfluidics arrays as disclosed in International Application No. WO97/21090.

For the purposes of this invention, the terms "capillary", "microcapillary and "microchannel" will be understood to be interchangeable and to be constructed of either wetting or non-wetting materials where appropriate.

For the purposes of this invention, the term "fluid chamber" will be understood to mean a defined volume on a rotor or microsystems platform of the invention comprising a fluid.

For the purposes of this invention, the term "entry port" will be understood to mean a undefined volume on a rotor or microsystems platform of the invention comprising a means for applying a fluid to the rotor or platform.

For the purposes of this invention, the term "capillary junction" will be understood to mean a junction of two components wherein one or both of the lateral dimensions of the junction are larger than the corresponding dimensions of the capillary. In wetting or wettable systems, such junctions are where capillary valving occurs, because fluid flow through the capillaries is stopped at such junctions. In non-wetting or non-wettable junctions, the exit from the chamber or reservoir is where the capillary junction occurs. In general, it will be understood that capillary junctions are formed when the dimensions of the components change from a small diameter (such as a capillary) to a larger diameter (such as a chamber) in wetting systems, in contrast to non-wettable systems, where capillary junctions form when the dimensions of the components change from a larger diameter (such as a chamber) to a small diameter (such as a capillary).

For the purposes of this invention, the term "biological sample" or "biological fluid

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sample" will be understood to mean any biologically-derived analytical sample, including but not limited to blood, plasma, serum, lymph, saliva, tears, cerebrospinal fluid, urine, sweat, plant and vegetable extracts, semen, cell culture fluids, cellular lysate aqueous or non-aqueous fractions of the above and ascites fluid.

5   For the purposes of this invention, the term "air displacement channels" will be understood to include ports in the surface of the platform that are contiguous with the components (such as chambers and reservoirs) on the platform, and that comprise vents and microchannels that permit displacement of air from components of the platforms and rotors by fluid movement.

For the purposes of this invention, the term "capillary action" will be understood to mean fluid flow in the absence of rotational motion or centripetal force applied to a fluid on a rotor or platform of the invention.

For the purposes of this invention, the term "capillary microvalve" will be understood to mean a capillary comprising a capillary junction whereby fluid flow is impeded and can be motivated by the application of pressure on a fluid, typically by centripetal force created by rotation of the rotor or platform of the invention.

15   The microplatforms of the invention (preferably and hereinafter collectively referred to as discs or disks for the purposes of this invention), the terms "microplatform", "microsystems platform" and "disc" or "disk" are considered to be interchangeable, and are provided to comprise one or a multiplicity of microsynthetic or microanalytic systems. Such microsynthetic or microanalytic systems in turn comprise combinations of related components as described in further detail herein that are operably interconnected to allow fluid flow between components upon rotation of the disk. These components can be fabricated as described below either integral to the disk or as modules attached to, placed upon, in contact with or embedded in the disk. The invention also comprises a micromanipulation device for manipulating the disks of the invention, wherein the disk is rotated within the device to provide centripetal force to effect fluid flow on the disk. Accordingly, the device provides means for rotating the disk at a controlled rotational velocity, for stopping and starting disk rotation, and advantageously for changing the direction of rotation of the disk. Both electromechanical means and control means, as further described herein, are provided as components of the devices of the invention. User interface means (such as a keypad and a display) are also

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provided, as further described in International Application WO97/21090.

Fluid (including reagents, samples and other liquid components) movement is controlled by centripetal acceleration due to rotation of the platform. The magnitude of centripetal acceleration required for fluid to flow at a rate and under a pressure appropriate for a particular microsystem is determined by factors including but not limited to the effective radius of the platform, the position angle of the structures on the platform with respect to the direction of rotation and the speed of rotation of the platform.

The capillary junctions and microvalves of the invention are based on the use of rotationally-induced fluid pressure to overcome capillary forces. Fluids which completely or partially wet the material of the microchannels (or reservoirs, reaction chambers, detection chambers, etc.) which contain them experience a resistance to flow when moving from a microchannel of narrow cross-section to one of larger cross-section, while those fluids which do not wet these materials resist flowing from microchannels (or reservoirs, reaction chambers, detection chambers, etc.) of large cross-section to those with smaller cross-section. This capillary pressure varies inversely with the sizes of the two microchannels (or reservoirs, reaction chambers, detection chambers, etc., or combinations thereof), the surface tension of the fluid, and the contact angle of the fluid on the material of the microchannels (or reservoirs, reaction chambers, detection chambers, etc.) . Generally, the details of the cross-sectional shape are not important, but the dependence on cross-sectional dimension results in microchannels of dimension less than 500μm exhibit significant capillary pressure. By varying the intersection shapes, materials and cross-sectional areas of the components of the microsystems platform of the invention, valves are fashioned that require the application of a particular pressure on the fluid to induce fluid flow. This pressure is applied in the disks of the invention by rotation of the disk (which has been shown above to vary with the square of the rotational frequency, with the radial position and with the extent of the fluid in the radial direction). By varying capillary valve cross-sectional dimensions as well as the position and extent along the radial direction of the fluid handling components of the microsystem platforms of the invention, capillary valves are formed to release fluid flow in a rotation-dependent manner, using rotation rates of from 100rpm to several thousand rpm. This arrangement allows complex, multistep fluid processes to be carried out using a pre-determined, monotonic increase in rotational rate. The

theoretical principles underlying the use of capillary junctions and microvalves are disclosed in International Patent Application, Publication No. WO98/07019, incorporated by reference.

The instant invention provides microsystems platforms comprising microfluidics components, heating elements, temperature sensing elements, capillary valves, sacrificial valves and a rotor design for transmitting electrical signals to and from the microsystems platforms of the invention. The invention provides fluidics components for capillary metering of precise amounts of a volume of a fluid sample from the application of a less precise volume of a fluid sample at an entry port on the microsystem platform. These embodiments of the invention provide for delivery of precise amounts of a sample such as a biological fluid sample without requiring a high degree of precision or accuracy by the operator or end-user in applying the fluid to the platform, and is advantageous in embodiments of the microsystems platforms of the invention that are used by consumers and other relatively unsophisticated users. The invention also provides laminar flow-dependent replacement of a fluid in a first chamber by a second displacement fluid in a second chamber on the platform. These embodiments of the invention provide approximately complete replacement of a fluid in one chamber on the platform with fluid from another, and thereby provide means for practicing sequential chemical reactions and other sequential processes on the platform under conditions wherein mixing of the two fluids is disadvantageous. The invention also provides turbulent flow mixing components, which permit thorough mixing of different fluid components on the platform, and in particular, the invention provides mixing chambers fluidly connected with fluid reservoirs containing equal amounts of two or more different fluids or unequal amounts of two or more different fluids. In addition, the invention provides fluid reservoirs fluidly connected with mixing chamber of the invention and shaped to determine the relative rate of flow of each of the different fluids into the mixing chamber. In these embodiments, gradients of two fluids differing in viscosity, solute concentration or concentration of suspended particulates can be produced using the mixing chambers of the invention, as disclosed; USSN 09/083,678 incorporated by reference. Such gradients can be transferred to reservoirs on the platform for further analytical manipulations, and can form the basis for controlled testing of concentration-dependent effects of various catalysts, drugs, toxins or other biological or chemical agents.

Platforms of the invention such as disks and the components comprising such platforms are

advantageously provided having a variety of composition and surface coatings appropriate for a particular application. Platform composition will be a function of structural requirements, manufacturing processes, and reagent compatibility/chemical resistance properties. Specifically, platforms are provided that are made from inorganic crystalline or amorphous materials, *e.g.* silicon,

5 silica, quartz, inert metals, or from organic materials such as plastics, for example, poly(methyl methacrylate) (PMMA), acetonitrile-butadiene-styrene (ABS), polycarbonate, polyethylene, polystyrene, polyolefins, polypropylene and metallocene. These may be used with unmodified or modified surfaces. Surface properties of these materials may be modified for specific applications. Surface modification can be achieved by silanization, ion implantation and chemical treatment with

10 inert-gas plasmas (*i.e.*, gases through which electrical currents are passed to create ionization). Also provided by the invention are platforms made of composites or combinations of these materials, *for example*, platforms manufactures of a plastic material having embedded therein an optically transparent glass surface comprising for example the detection chamber of the platform. Microplatform disks of the invention are preferably fabricated from thermoplastics such as teflon,

15 polyethylene, polypropylene, methylmethacrylates and polycarbonates, among others, due to their ease of molding, stamping and milling. Alternatively, the disks can be made of silica, glass, quartz or inert metal. A fluid handling system is built by sequential application of one or more of these materials laid down in stepwise fashion onto the thermoplastic substrate. Alternatively, the entire disc can be injection molded, embossed or stamped. Disks of the invention are fabricated with an

20 injection molded, optically-clear base layer having optical pits in the manner of a conventional compact disk (CD). The optical pits provide means for encoding instrument control programming, user interface information, graphics, data analysis, and sound specific to the application and driver configuration. The driver configuration depends on whether the micromanipulation device is a hand-held, benchtop or floor model, and also on the details of external communication and other specifics

25 of the hardware configuration. This layer is then overlaid with a reflective surface, with appropriate windows for external detectors, specifically optical detectors, being left clear on the disk. Other layers of polycarbonate of varying thickness are laid down on the disk in the form of channels, reservoirs, reaction chambers and other structures, including provisions on the disk for valves and other control elements. These layers can be pre-fabricated and cut with the appropriate geometries

for a given application and assembled on the disk. Layers comprising materials other than polycarbonate can also be incorporated into the disk. The composition of the layers on the disk depend in large part on the specific application and the requirements of chemical compatibility with the reagents to be used with the disk. Electrical layers can be incorporated in disks requiring electric

5   circuits, such as electrophoresis applications and electrically-controlled valves. Control devices, such as integrated circuits, laser diodes, photodiodes and resistive networks that can form selective heating areas or flexible logic structures can be incorporated into appropriately wired recesses, either by direct fabrication of modular installation onto the disk. Reagents that can be stored dry can be introduced into appropriate open chambers by spraying into reservoirs using means similar to inkjet

10   printing heads, and then dried on the disk. A top layer comprising access ports and air vents, ports or shafts is then applied. Liquid reagents are then injected into the appropriate reservoirs, followed by application of a protective cover layer comprising a thin plastic film.

The platforms of the invention are preferably provided with a multiplicity of components, either fabricated directly onto the platform, or placed on the platform as prefabricated modules. In

15   addition to the integral components, certain devices and elements can be located external to the platform, optimally positioned on a device of the invention in relation to the platform, or placed in contact with the platform either while rotating or when at rest. Components optimally comprising the platforms of the invention or a controlling device in combination therewith include detection chambers, reservoirs, valving mechanisms, detectors, sensors, temperature control elements, filters,

20   mixing elements, and control systems.

This invention provides microsystems platforms comprising the following components.

1.   Fluidics Components

The platforms of the invention are provided comprising microfluidics handling structures in

25   fluidic contract with one another. In preferred embodiments, fluidic contact is provided by capillary or microchannels comprising the surface of the platforms of the invention. Microchannel sizes are optimally determined by specific applications and by the amount of delivery rates required for each particular embodiment of the platforms and methods of the invention. Microchannel sizes can range from 0.02mm to a value close to the thickness of the platform. Microchannel shapes can be

trapezoid, circular or other geometric shapes as required. Microchannels preferably are embedded in a platform having a thickness of about 0.1 to 100mm, wherein the cross-sectional dimension of the microchannels across the thickness dimension of the platform is less than 500μm -800μm and from 1 to 90 percent of said cross-sectional dimension of the platform. In these embodiments, which

5   is based on the use of rotationally-induced fluid pressure to overcome capillary forces, it is recognized that fluid flow is dependent on the orientation of the surfaces of the components. Fluids which completely or partially wet the material of the microchannels, reservoirs, detection chambers, etc. (i.e., the components) of the platforms of the invention which contain them experience a resistance to flow when moving from a component of narrow cross-section to one of larger cross-

10   section, while those fluids which do not wet these materials resist flowing from components of the platforms of the invention of large cross-section to those with smaller cross-section. This capillary pressure varies inversely with the sizes of the two components, or combinations thereof, the surface tension of the fluid, and the contact angle of the fluid on the material of the components . Generally, the details of the cross-sectional shape are not important, but the dependence on cross-sectional

15   dimension results in microchannels of dimension less than 500μm exhibit significant capillary pressure. By varying the intersection shapes, materials and cross-sectional areas of the components of the platform of the invention, "valves" are fashioned that require the application of a particular pressure on the fluid to induce fluid flow. This pressure is applied in the disks of the invention by rotation of the disk (which has been shown above to vary with the square of the rotational frequency,

20   with the radial position and with the extent of the fluid in the radial direction). By varying capillary valve cross-sectional dimensions as well as the position and extent along the radial direction of the fluid handling components of the platforms of the invention, capillary valves are formed to release fluid flow in a rotation-dependent manner, using rotation rates of from 100rpm to several thousand rpm. This arrangement allows complex, multistep fluid processes to be carried out using a pre-

25   determined, monotonic increase in rotational rate.

     A first example of the microfluidics arrays provided by this invention is shown in Figures 1A through 1D. A microsystems platform is provided by the invention that is specifically designed for performing an assay for detecting a chemical species in a complex mixture, preferably an aqueous mixture. These Figures illustrate an array advantageously used for any such assay;

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detection of blood glucose concentration is illustrated herein. It will be understood that in this description, the use of the words "cell" and "particulate" will be interchangeable, and that cells are a particular example of a specific particulate species comprising the fluid samples, and most preferably the biological fluid samples, analyzed using the present invention.

5   A microsystems platform provided by the invention and specifically designed for performing blood glucose assay is illustrated in Figure 1A. Disk embodiments of the platforms of the invention were fashioned, *for example*, from machined acrylic or injection-molded polycarbonate, polystyrene, polypropylene, acetonitrile-butadiene-styrene, or high-density polyethylene (HDPE). The overall disc dimensions include an outer radius of from about 1cm to about 15cm and an inner

10   radius of from about 0.1cm to about 1cm, wherein the disk was mounted on the spindle of a rotary device. The thickness of the disc ranged from about 0.2mm to about 1.5cm. All surfaces coming into contact with blood on the platform may be advantageously treated with heparin, EDTA or other anticoagulants to facilitate fluid flow thereupon

The components of the blood glucose assay were prepared as follows. Fluid sample entry

15   port **101** having a depth in the platform surface from about 0.15cm to about 3mm and lateral dimensions of from about 0.1cm to about 2.5cm were constructed on the platform, and designed to accommodate a volume of from about 5 to about 200 μL. This entry port was fluidly connected with a metering capillary **102** having a square cross-sectional diameter of from about 0.02mm to about 2cm and proximal ends rounded with respect to entry port **101**; the length of this metering chamber

20   array was sufficient to contain a total volume of from about 15 to about 150μL. The entry port was also constructed to be fluidly connected with an overflow capillary **121** having a cross-sectional diameter of from about 0.02mm to about 2mm and proximal ends rounded with respect to entry port **101**. The overflow capillary was fluidly connected with an overflow chamber **122** having a depth in the platform surface of from about 0.02mm to about 9mm, greater than the depth of the overflow

25   capillary **121**. Each of the chambers on the platform were also connected with air ports or air channels, such as **114**, that have dimensions of from about 0.02mm to about 2mm deep and permitted venting of air displaced by fluid movement on the platform. A capillary junction **115** that is from about 0.03mm to about 2.2mm deep is present in the air channel to prevent fluid flow into the air channel.

Entry port **101** was positioned on the platform from about 1cm to about 12cm from the center of rotation. Metering chamber **102** extended from about 0.25cm to about 2cm from entry port **101**. The extent of the length of overflow capillary **121** was greater than the extent of the length of metering capillary **102**. The position of overflow chamber **122** was, *for example*, from about 1.2cm to about 14cm from the axis of rotation.

In an alternative embodiment of the fluid metering structures of the invention, shown in Figure 1B, fluid sample entry port **101** is comprised of a funnel having a depth in the platform surface from about 0.75mm to 5mm, lateral dimensions of from about 2mm to 2cm in the long dimension and from about 2mm to 2cm in the short dimension and positioned from about 0.75cm to about 5cm from the center of rotation, and designed to accommodate a volume of about 5 to about 200 μL. The bottom surface of the entry port consists of a slot **120** fluidly connected to microfluidics structures formed in the other (under) side of the microfluidics surface of the platform. Entry port **101** is connected to entry passageway **102** having a rectangular cross-sectional diameter of from about 0.1mm to 5mm wide and from about 0.1mm to 5mm deep and extending from about 0.1cm to about 3cm from entry port **101**. The entry passageway **102** is fluidly connected to blood entry chamber **103**. Blood entry chamber **103** has a depth of from about 0.1mm to 5mm, lateral dimensions of from about 1mm to about 4cm, and is positioned from about 0.75cm to about 2.5cm from the center of rotation. Blood entry chamber **103** further comprises blood metering volume **104** designed to accommodate a volume of from about 1μL to about 50μL and an overflow passageway **105**. The overflow passageway was fluidly connected with an overflow capillary **106** having a depth in the platform surface of from about 0.1mm to about 1mm. Overflow capillary **121** is further fluidly connected with overflow chamber **122** which is comprised of two parts, a shallow outer portion **108** having a depth from about 0.05mm to about 0.5mm and a deeper inner portion **109** having a depth of from about 0.1mm to 5mm. Overflow chamber **122** is positioned from about 4cm to about 5.8cm from the axis of rotation. The distal end of the blood overflow capillary **121** is chosen to be farther from the center of rotation than the distal end of blood chamber **104**. Each of the chambers on the platform were also connected with air ports or air channels **114**, that are from about 0.1mm to about 5mm deep and permit venting of air displaced by fluid movement on the platform. A capillary junction **115** that is from about 0.03mm to about 2.2mm deep is present in the

air channel to prevent fluid flow into the air channel. In alternative embodiments, these vents may be multiply connected to one another through a manifold such that fluid flow merely displaces air within the structure, rather than forcing it through vents in the platform surface.

Alternatively, an unmetered amount of a blood sample is placed directly in blood fluid chamber **104**, which in this embodiment is open to the surface of the disk to accept blood application. In these embodiments, the amount of blood fluid to be assayed is controlled by the capacity of assay chamber **107** or the matrix **106** contained therein as described below.

As described herein for performing blood glucose assays (and as understood in the art that essentially the same microfluidics structures can be used for a multiplicity of blood analyte assays or, more generally, for analyte assays in any fluid sample, most preferably a biological fluid sample), a capillary barrier prevents movement of the fluid sample directly into the assay chamber **107**. In the metering structure shown in Figure 1A, fluid (or more properly for this illustrative example, blood) metering capillary **102** acted as a capillary barrier that prevented blood fluid flow from metering capillary **102** at a first, non-zero rotational speed f_1, ranging from about 200rpm to about 450rpm and sufficient to permit fluid flow comprising overflow from the entry port **101** through overflow capillary **121** and into overflow chamber **122**. This capillary boundary was constructed to be overcome at a second rotational speed f_2, ranging from about 250rpm to about 900rpm (where $f_2 > f_1$). In the alternative embodiments shown in Figures 1B and 1C, blood fluid chamber **104** acted as a capillary barrier that was maintained during rotation at a rotational speed sufficient to motivated excess fluid sample from the entry port **101** to the overflow chamber **122**, and was overcome at a second rotational speed greater than the first rotational speed to permit fluid sample flow into assay chamber **107**.

Blood metering capillary **102** and blood fluid chamber **104** were in different alternatives of the microsystems platforms of the invention fluidly connected to capillary **110** that was from about 0.02mm to about 2mm deep and had a cross-sectional diameter of from about 0.02mm to about 2mm and was connected to capillary or sacrificial valve **111**. Sacrificial or capillary valve **111** was further fluidly connected with capillary **112** that was from about 0.02mm to about 2mm deep and had a cross-sectional diameter of from about 0.02mm to about 2mm, and further to assay chamber **107**. In capillary valve embodiments, the junction between capillary **110** and capillary **112** creates

capillary valve **111**, wherein said capillary valve **111** was from about 0.03mm to about 2.2mm deep and had a cross-sectional diameter of from about 0.03mm to about 2.2mm. In order to function as a capillary valve, the junction between capillary **110** and capillary **112** must have a depth and/or cross sectional area greater than that of capillary **110** in a disc fabricated from hydrophilic materials such as acrylic. In sacrificial valve embodiments, intermediate melting temperature materials (including, *for example*, waxes as described above) are placed in the lumen of capillary **110** forming a fluid-tight seal. In these embodiments, chamber **111** is fluidly connected to the sacrificial valve so that melted wax from the release sacrificial valve is sequestered in the chamber.

Rectangular assay chamber **107** was constructed in the surface of the platform to have a depth of from about 0.2mm to about 3mm, most preferably comprising a circular or rectangular depression **113** connected to capillary **112**. Depression **113** was constructed to have a volumetric capacity of from half to twice the assay volume. Assay chamber **107** also comprised a pad or matrix **106** of a hydrophilic substance. Materials used to prepare said matrices include but are not limited to derivatized nylons, nitrocellulose, fiberglass and polyesters, most preferably having a pore size of 0.2 - 2.0μm, most preferably comprising a positively-charged nylon matrix having a pore size of about 0.8μm. The upper limit on pore size of matrix **106** is chosen to inhibit or prevent blood cell entry into the matrix. The matrix is positioned in assay chamber **107** to be in fluidic contact with depression **113**, more preferably covering depression **113**, and most preferably having a surface area greater than the surface area of depression **113**. The matrix was further impregnated with immobilized reagents which produce a detectable product proportional to the amount or concentration of glucose in a blood sample. Most preferably, the detectable product is a colored product, *i.e.*, a product absorbing light at a detectable, most preferably a visible, wavelength.

In preparing matrices according to the invention, reagents advantageously used to detect and more preferably quantitate an amount of a component of a biological fluid sample are impreganted into the matrix. As a non-limiting example, glucose is detected according to the invention using a glucose oxidase assays system, as described in additional detail below. Matrices for performing such assays using the microfluidics platforms of the invention are prepared by saturating the matrix membrane with an 8mL solution of distilled water containing 0.12g 2,5-ferandione polymer with methoxylene (CAS: 9011-16-9), 10mg EDTA, 200mg Polypep® Low Viscosity (Sigma Chemical

Company, St. Louis, Missouri), 668mg sodium citrate, 28.75mg glucose oxidase and 27.3mg peroxidase. The saturated membrane is then dried and then saturated with 5mL acetonitrile and 5mL distilled water containing 40mg 3-methyl-2-benzothiazolinone hydrazone and 80mg 3-dimethylaminobenzoic acid. The saturated membrane is dried and applied to the microfluidics disk

5 of the invention for use.

In an alternative embodiment shown in Figure 15, assay chamber **107** is comprised of a rectangular cavity in the surface of the platform having a depth of from about 0.2mm to about 3mm which is fluidly connected at its end proximal to the axis of rotation to capillary **112** and to air displacement channels **114** and ports **115**. The second member of the assay chamber is a

10 rectangular piece made from the same material as the platform or other material and designed to snap into the cavity forming liquid-tight seals around all edges. The snap-in piece has two faces, an A face and a B face. The A face consists of a fluid entry channel **112** connected to depression **113**; depression **113** is further connected to air displacement channels **114**. Depression **113** is from about 0.05mm to about 5mm deep and has a dimensions from about 0.5mm by 4mm, having a volumetric

15 capacity of from about half to about twice the assay volume applied to the disc. A pad or matrix **106** is attached to the A face of the snap-in piece, comprising a hydrophilic substance possessing a pore size of 0.2 - 2.0μm, most preferably comprising a positively-charged nylon matrix having a pore size of about 0.8μm. The upper limit on pore size of matrix **106** is chosen to inhibit or prevent blood cell entry into the matrix.. The matrix is positioned in assay chamber **107** to be in fluidic contact with

20 depression **113**, more preferably covering depression **113**, and most preferably having a surface area greater than the surface area of depression **113**. The matrix is further impregnated with immobilized reagents which produce a detectable product proportional to the amount or concentration of glucose in a blood sample. Most preferably, the detectable product is a colored product, *i.e.*, a product absorbing light at a detectable, most preferably a visible, wavelength.

25 As illustrated in Figures 2A through 2F, in the use of this platform an imprecise volume (ranging from 20-150μL) of blood was applied to the entry port **101**. In embodiments of the platform comprising air displacement channels, the fluid wicked into air channel **114** and was stopped by capillary junction **115**. Fluid also wicked into metering capillary **102** and overflow capillary **121**. Fluid flowed through the metering capillary **102** and overflow capillary **121** at no

rotational speed until the fluid reached capillary junctions at the junction between metering capillary 102 and capillary 110 and overflow capillary 121 and overflow chamber 122. Metering capillary 102 was constructed to define a precise volume from about 15 to about 60μL of fluid between entry port 101 and capillary junction 111, which was designed to be at least the amount of the fluid placed by the user in entry port 101.

After sample loading by a user and filling of metering capillary 102 and overflow capillary 121 at no rotational speed, the platform was spun at a first rotational speed f_1, ranging from about 50rpm to about 600rpm, which was sufficient to motivate fluid flow through the overflow capillary 121 in this microfluidics array having an entry port 101 with a depth of from about 0.2mm to about 3mm, metering capillary 102 with dimensions of about 0.5mm x 0.5mm in cross-section and a length of about 2.2-3.8cm from the center of rotation and an overflow capillary 121 with dimensions of about 0.5mm x 0.5mm in cross-section and a length of about 5.4cm from the center of rotation.

Due to the greater distance of the end of overflow capillary 121 from the center of rotation than the end of metering capillary 102, at rotational speed f_1 fluid flowed through overflow capillary 121 into overflow chamber 122. The platform was spun until all excess fluid is evacuated from entry port 101 and into overflow chamber 122, except the fluid contained in metering chamber102.

At a second rotational speed f_2 of from about 100rpm to about 1000rpm, the precise amount of fluid contained in metering capillary 102 was delivered into assay chamber 107. In embodiments comprising a sacrificial valve 111 in-line with capillary 110 at a position between capillary 110 and 112 shown in Figure 2B, release of the sacrificial valve resulted in fluid flow through capillary 112 and into assay chamber 107. In said embodiments, fluid flow is achieved at rotational speed f_2 with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve 111 at a position between capillary 110 and 112 shown in Figure 2B, capillary 110 preferably filled along with filling of metering capillary 102 until blood reached capillary junction 111 at the junction between capillary 110 and capillary 112; in such embodiments, the capillary junction had a depth of from about 0.03mm to about 2.2mm, or at least greater than the depth of capillary 110.

Blood flowing into assay chamber 107 is preferentially directed to depression 113 in the assay chamber; the dimensions of depression 113 are conveniently chosen to be able to contain

substantially all of the blood fluid of the sample metered through metering capillary **102** into assay chamber **107** (Figure 2C). Displaced air flows through air channel **114**, and may be vented to the surface of the disc or in communication with blood fluid chamber **104**.

As blood flows into depression **113**, the fluid component of the blood is driven by pressure and hydrophilic forces into matrix **106**; the pore size of the matrix is chosen to prevent the cellular components of the blood from entering the matrix (Figure 1.4). In preferred embodiment, the cellular component of the blood is retained in depression **113** and the fluid component is efficiently distributed by wicking and by centripetal force into matrix **106**. In an alternative embodiment, alternative matrix **118** comprises at least two distinct elements that are compressed or adhered to one another in the assays chamber. The first element is similar to the reagent-containing matrix **106** described above; however, this embodiment of the matrix has a pore size that is not limited by the size of cellular components of the blood, and can be any pore size deemed optimal on experimental, economic, manufacturing or availability grounds. The second element comprises a filtering layer having a pore size that prevents cells and cellular debris from entering this portion of the matrix. In a preferred embodiment, the two elements and rearranged in assay chamber **107** so that second matrix element is in contact with depression **113** wherein the blood aliquot is first contacted with the second matrix component. Blood fluid flows into and through the second matrix element and into the first matrix element, whereby cellular components of blood are prevented from entering assay chamber **107** by the pore size of second matrix element. Preferably, the dimensions of the matrix element is about 1cm by about 0.75cm and has a thickness of about 0.05cm.

As the fluid component of the blood wicks into matrix **106**, dried reagents are solubilized and the reaction of the blood component catalyzed by said reagents proceeds. The timescale over which these chemical reactions take place is chosen to be long compared with the time it takes for the fluid component of blood to saturate the matrix **106**. The time it takes for the blood fluid to saturate the matrix is dependent on the capacity of the matrix **106** to absorb the fluid and the delivery speed of the blood fluid to the assay chamber **107**, which in turn is dependent on the rotational velocity and the cross-sectional dimensions of capillary **110**. In preferred embodiments, the reaction(s) goes to completion within about 1min. Reaction of the blood component(s) with the reagents produce

colored product which is then detected (Figure 2E). In preferred embodiments, detection is performed spectrophotometrically, including absorbance, transmittance, reflection, fluorescence, and chemiluminescence, although visual inspection is also contemplated in alternative embodiments of the invention.

5   In an alternative embodiment, assay chamber 107 further comprises a detection cell 117 that is laterally adjacent to the portion of assay chamber 107 comprising depression 113 (shown in Figures 3A through 3E). This embodiment uses a variation of alternative matrix 118 wherein the matrix comprises a blood separation element and a blood fluid wicking element. These elements are arranged in assay chamber 107 so that the blood separation element is in contact with depression 113 and the blood fluid wicking element is compressed or attached to the blood separation element along its entire extent. A blood fluid wicking element having dimensions as described above and sufficient to encompass the entire surface extent of the blood separation element and to further extend throughout the complete extent of assay chamber 107, including detection cell 117. Reagents are only present in the portion of the blood fluid wicking element in that portion of the element comprising detection cell 117; this arrangement is shown in detail in Figure 3A.

In the practice of this embodiment of the invention, blood delivered to depression 113 wicks through the blood separation element and into the blood fluid wicking element (Figure 3B and 3C). As the region of blood fluid wicking element above depression 113 saturates with blood plasma, the blood fluid wicks laterally into the portion of the matrix 118 comprising detection cell 117 (Figure 3D). Wetting of this portion of the matrix, which comprises the immobilized reagents and initiates the glucose detection reaction(s) as described in Example 1, with the production of a colored product (Figure 3E). The amount of colored product produced is detected and the amount of glucose in the blood sample determined thereby.

Another alternative embodiment of a blood glucose assay microsystems platform is shown in Figures 4A through 4E. Construction of the disk embodiments of the platforms of the invention were as described above.

The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber 201,

metering capillary **202**, overflow capillary **203**, and overflow chamber **205**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **214**, and capillary junction(s) **215**, that permit venting of air displaced by fluid movement on the platform.

Metering capillary **202** is fluidly connected to capillary **210** that was from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to capillary or sacrificial valve **211**. Sacrificial or capillary valve **211** is further fluidly connected with capillary **212** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm; capillary **212** is further fluidly connected to assay chamber **207**. Where capillary **210** is connected with capillary valve **211**, said capillary valve **211** is from about 0.03mm to about 2.2mm deep and has a cross-sectional diameter of from about 0.03mm to about 2.2mm.

Assay chamber **207** comprises a depression in the surface of the platform having a depth of from about 0.2mm to about 3mm preferably comprising a circular or rectangular depression **213** connected to capillary **212** and most preferably wherein a metered or otherwise controlled amount of blood can be contained in the assay chamber. Assay chamber **207** also comprises a pad or matrix **206** of a hydrophilic substance possessing a pore size of from about 0.2 to about 2μm, most preferably about 0.8μm. The upper limit on pore size of matrix **206** is chosen to inhibit or prevent blood cell entry into the matrix, and to promote entry or wicking of the fluid component of blood (plasma or serum) to enter the body of the matrix. The matrix is positioned in assay chamber **207** to be in fluidic contact with depression **213**, more preferably covering depression **213**, and most preferably having a surface area greater than the surface area of depression **213**; in embodiments not having depression **213** as a component of assay chamber **207**, the matrix substantially fills the volumetric extent of the assay chamber. The matrix is further impregnated with immobilized reagents which produce a detectable product proportional to the amount or concentration of glucose in a blood sample. Most preferably, the detectable product is a colored product, *i.e.*, a product absorbing light at a detectable, most preferably a visible, wavelength.

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In an alternative embodiment, assay chamber **207** comprises a surface wherein reagents **219** are directly dried or otherwise immobilized on the surface of the chamber, and in such embodiments the assay chamber does not comprise matrix **206**.

Assay chamber **206** is further fluidly connected with capillary **220** at a position preferably most distal to the axis of rotation. Capillary **220** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm, and is further fluidly connected with waste reservoir **221**. Waste reservoir **221** is positioned from about 1.2 cm to about 14cm from the axis of rotation, and has a depth in the platform of from about 0.2mm to about 3mm. Assay chamber **206** is also fluidly connected with capillary **222** at a position preferably most proximal to the axis of rotation. Capillary **222** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm, and is further fluidly connected with wash buffer reservoir **223**. Wash buffer reservoir **223** has a depth in the platform of from about 0.2mm to about 3mm and is positioned from about 1.2cm to about 14cm from the axis of rotation, and in any event more proximal to the axis than assay chamber **206**. Fluid flow from wash buffer reservoir **223** through capillary **222** is controlled by either capillary valve **224** or sacrificial valve **225**. Where capillary **222** was connected with capillary valve **224**, said capillary valve **224** is from about 0.03mm to about 2.2mm deep and has a cross-sectional diameter of from about 0.03mm to about 2.2mm.

As illustrated in Figures 4A through 4D, in the use of this platform an imprecise volume (ranging from 20-150μL of fluid) of blood is applied to the entry port **201**. Application of blood to the platform and delivering a metered amount of blood to metering capillary **202** was achieved as described above. Alternatively, an unmetered amount of blood is introduced onto the platform directly into assay chamber **207**. Preferably, from about 15μL to about 150μL of blood is delivered to assay chamber **207**.

At a rotational speed f_2 of about 100-1000rpm, the precise amount of fluid contained in metering capillary **202** is delivered into assay chamber **207**. In embodiments comprising a sacrificial valve **211** in-line with capillary **210** at a position between capillary **210** and assay chamber **207** as shown in Figure 3.1, release of the sacrificial valve results in fluid flow through capillary **212** and into assay chamber **207**. In said embodiments, fluid flow is achieved at rotational speed f_2 with

removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve 211 at a position between capillary 210 and 212 as shown in Figure 3B, capillary 210 preferably fills along with filling of blood metering capillary 202 until blood reaches capillary junction 211 at the junction between capillary 210 and capillary 212; in such embodiments, the capillary junction had a depth of from about 0.03mm to about 2.2mm. At a rotational speed f_2 of about 100-1000rpm, the fluid contained in blood metering capillary 202 is delivered into assay chamber 207 (Figure 3C).

Blood flowing into assay chamber 207 is directed to matrix 206 in the assay chamber; the dimensions of matrix 206 are conveniently chosen to be able to contain substantially all of the blood fluid of the sample metered through metering capillary 202 into assay chamber 207. Displaced air flows through air channel 214, and may be vented to the surface of the disc or in communication with blood metering capillary 202 or entry port 201. Alternatively, in embodiments wherein the blood sample is unmetered, the capacity of the matrix 206 is sufficient to absorb a controlled amount of blood. In further alternative embodiments, wherein assay chamber does not comprise matrix 206, the assay chamber is provided having a capacity for a controlled volume of blood.

As blood flows into assay chamber 207, the fluid component of the blood wicks into matrix 206; the pore size of the matrix is chosen to prevent the cellular components of the blood from entering the matrix. In preferred embodiment, the cellular component of the blood is retained in assay chamber 207 and the fluid component is efficiently wicked uniformly throughout matrix 206.

As the fluid component of the blood wicks into matrix 206, dried reagents are solubilized and the reaction of the blood component catalyzed by said reagents proceeds. The timescale over which these reactions take place is chosen to be long compared with the time it takes for the fluid component of blood to saturate the matrix 206; however, in preferred embodiments, the reaction(s) goes to completion within about 0.5 to about 5min. Reaction of the blood component(s) with reagents produce colored product which is then detected. In preferred embodiments, detection is performed spectrophotometrically, although visual inspection is also contemplated in alternative embodiments of the invention.

After a time sufficient to produce a detectable amount of a colored product, the wash buffer is released from wash buffer reservoir **223** through capillary **222** and into assay chamber **207**. In embodiments comprising a sacrificial valve **225** in-line with capillary **222** at a position between capillary **222** and assay chamber **207** shown in Figure 3.3, release of the sacrificial valve results in fluid flow through capillary **222** and into assay chamber **207**. In said embodiments, fluid flow is achieved at rotational speed f_3 of about 250rpm to about 1500rpm with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve **224** at a position between capillary **222** and assay chamber **207** shown in Figure 3D, capillary **222** preferably fills along with filling of blood fluid chamber **204** until blood reached capillary junction **224**; in such embodiments, the capillary junction had a depth of from about 0.03mm to about 2.2mm. At a higher rotational speed f_4 of about 400-2000rpm, the fluid contained in wash reservoir **223** is delivered into assay chamber **207** (Figure 3D). Because the fluid flow of wash buffer into the assay chamber and fluid from the assay chamber to the waste chamber is laminar, there is very little mixing of the washing fluid with the fluid initially in the assay chamber. The wash fluid displaces the fluid sample in the assay by pushing it into the waste chamber. The exit of capillary **220** into chamber **221** is at a radial position such that assay chamber **207** must remain filled with fluid during this washing process. The quality of fluid removal is such that no more than 1 part in 1000 of the fluid in the chamber **207** (which has not been imbibed into matrix **206**) remains. The fluid which has wicked into matrix **206** is not removed during this wash because of the small pore size of the matrix which resists fluid flow; furthermore, color reagents do not diffuse out of matrix **206** if the wash time is relatively short (less than a few hundred seconds). As a result, interfering blood fluid components such as hemoglobin are removed from chamber **207** while substantially leaving behind color reagents in matrix **206**. These are then interrogated spectrophotometrically in assay chamber **207** (Figure 4E).

Another alternative embodiment of a blood glucose assay microsystems platform is shown in Figures 5A through 5E. Construction of the disc embodiments of the platforms of the invention were as described above.

The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber **301**, metering capillary **302**, overflow capillary **303**, and overflow chamber **305**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **314**, and

5   capillary junction(s) **315**, that permit venting of air displaced by fluid movement on the platform.

Metering capillary **302** is fluidly connected to capillary **310** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to capillary or sacrificial valve **311**. Sacrificial or capillary valve **311** is further fluidly connected with capillary **312** that is from about 0.02mm to about 2mm deep and has a cross-

10   sectional diameter of from about 0.02mm to about 2mm, and is further fluidly connected to assay chamber **307**. Where capillary **310** is connected with capillary valve **311**, said capillary valve **311** is from about 0.03mm to about 2.2mm deep and has a cross-sectional diameter of from about 0.03mm to about 2.2mm.

Assay chamber **307** comprises a pad or matrix **306** of a hydrophilic substance possessing a

15   pore size of from about 0.2µm to about 2µm, most preferably about 0.8µm. The upper limit on pore size of matrix **306** is chosen to inhibit or prevent blood cell entry into the matrix. The matrix is further impregnated with immobilized reagents which produce a detectable product proportional to the amount or concentration of glucose in a blood sample. Most preferably, the detectable product is a colored product, *i.e.*, a product absorbing light at a detectable, most preferably a visible,

20   wavelength.

As illustrated in Figures **5A** through **5E**, in the use of this platform an imprecise volume (ranging from about 15µL to about 150µL of fluid) of blood is applied to the entry port **301**. Application of blood to the platform and delivering a metered amount of blood to blood metering capillary **302** was achieved as described above. Alternatively, an unmetered amount of blood is

25   introduced onto the platform directly into assay chamber **307**, preferably, from about 15µL to about 150µL.

At a rotational speed f_1 of 100-1000rpm the precise amount of fluid contained in metering chamber **302** is delivered into assay chamber **307**. In embodiments comprising a sacrificial valve

311 in-line with capillary 310 at a position between capillary 310 and 312 shown in Figure 5A, release of the sacrificial valve results in fluid flow through capillary 312 and into assay chamber 307. In said embodiments, fluid flow is achieved at rotational speed f_1 with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve 311 at a position

5   between capillary 310 and 312 shown in Figure 5B, capillary 310 preferably fills along with filling of blood metering capillary 302 until blood reaches capillary junction 311 at the junction between capillary 310 and capillary 312; in such embodiments, the capillary junction has a depth of from about 0.03mm to about 2.2mm. At a higher rotational speed f_2 of about 250rpm to about 1500rpm, the fluid contained in blood metering capillary 302 is delivered into assay chamber 307 (Figure 5C).

10   Blood flows into assay chamber 307 with displaced air flowing through air channel 314, and may be vented to the surface of the disc or in communication with blood metering capillary 302 or entry port 301 (Figure 5C). As blood flows into assay chamber 307, the fluid component of the blood wicks into matrix 306; the pore size of the matrix is chosen to prevent the cellular components of the blood from entering the matrix. In an alternative embodiment, alternative matrix 318

15   comprises at least two distinct elements that are compressed or adhered to one another in the assays chamber. The first element is similar to the reagent-containing matrix 306 described above; however, this embodiment of the matrix has a pore size that is not limited by the size of cellular components of the blood, and can be any pore size deemed optimal on experimental, economic, manufacturing or availability grounds. The general dimensions of the matrix are equivalent to the

20   dimensions disclosed above, so that the matrices are advantageously standardized and interchangeable on the microsystems platforms of the invention. The second element comprises a filtering layer having a pore size that prevents cellular components from entering this portion of the matrix. In a preferred embodiment, these elements are arranged in assay chamber 307 so that the second matrix element is in contact with the surface of assay chamber 307 wherein the blood aliquot

25   is first contacted with the second matrix component. Blood fluid wicks into and through second matrix element and into the first matrix element, whereby cellular components of blood are prevented from entering assay chamber 307 by the pore size of second matrix element.

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As the fluid component of the blood wicks into matrix **306**, dried reagents are solubilized and the reaction of the blood component catalyzed by said reagents proceeds as described below. The timescale over which these reactions take place is chosen to be long compared with the time it takes for the fluid component of blood to saturate the matrix **306**; however, in preferred embodiments, the

5 reaction(s) goes to completion within about 0.5 to about 5min. Reaction of the blood component(s) with reagents produce colored product (Figure 5D). After sufficient time for the reaction to proceed, the platform is centrifuged at a speed of from about 800rpm to about 2000rpm, wherein said speed pellets the cellular component of the blood, particularly the red blood cell component thereof, to the "bottom" or most axially distal portion of assay chamber **307**. Detection of the colored product of

10 the glucose detecting reaction in matrix **306** is performed at a position in assay chamber **307** radially more proximal to the axis of rotation than the position to which the cellular fraction has been pelleted (Figure 5E). In preferred embodiments, detection is performed spectrophotometrically, although visual inspection is also contemplated in alternative embodiments of the invention. The amount of colored product produced is detected and the amount of glucose in the blood sample

15 determined thereby.

Another alternative embodiment of a blood glucose assay microsystems platform is shown in Figures 6A through 6E. Construction of the disk embodiments of the platforms of the invention were as described above.

The blood application and metering components and their dimensions and relationships to

20 one another are identical to those described above, comprising sample entry port chamber **401**, metering capillary **402**, overflow capillary **403**, and overflow chamber **405**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **414**, and capillary junction(s) **415**, that permit venting of air displaced by fluid movement on the platform.

Metering capillary **402** is fluidly connected to capillary **410** that is from about 0.02mm to

25 about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to capillary or sacrificial valve **411**. Sacrificial or capillary valve **411** is further fluidly connected with capillary **412** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **412** is further fluidly connected

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to cell separation chamber **407**. Where capillary **410** is connected with capillary valve **411**, said capillary valve **411** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm.

Cell separation chamber **407** comprised a depression in the surface of the platform having
5   a depth of from about 0.02mm to about 3cm, most preferably comprising a circular or rectangular depression **413** connected to capillary **412**. Cell separation chamber also comprises a filter **406** consisting of a porous material whose pores are sized (from about 0.2μm to about 2μm) to filter red blood cells. Filter **406** is in contact with and more preferably adhered to the surface of cell separation chamber **407** and most extends over depression **413**. Blood flowing into cell separation
10   chamber **407** is directed to depression **413** by capillary **412**. As depression **413** fills, serum is both wicked and driven by rotation-induced pressure through the filter **406** to the upper surface of cell separation chamber **407**, leaving red blood cells and other cellular components trapped beneath filter **406** in depression **413**. The volume of blood fluid at the upper surface of cell separation chamber **407** ranges from about 15μL to about 150μL.

15   Cell separation chamber **407** is fluidly connected at a radial position distal from the axis of rotation to capillary **420** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **420** is further fluidly connected with assay chamber **421** that is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from about 0.02mm to about 10cm. Assay chamber **421** comprises a pad or matrix **422** of a
20   hydrophilic substance possessing any convenient pore size, for example, a pore size of 0.2 - 2.0μm, such as a pore size of about 0.8μm. The matrix is further impregnated with immobilized reagents which produce a detectable product proportional to the amount or concentration of glucose in a blood sample. Most preferably, the detectable product is a colored product, *i.e.*, a product absorbing light at a detectable, most preferably a visible, wavelength.

25   As illustrated in Figures 6A through 6E, in the use of this platform an imprecise volume (ranging from 15 to about 150 μL of fluid) of blood is applied to the entry port **401**. Application of blood to the platform and delivering a metered amount of blood to metering capillary **402** was achieved as described in Example 1 above. At a rotational speed f_1 of 100-1000rpm, the precise

amount of fluid contained in metering capillary **402** is delivered into assay chamber **407**. Alternatively, an unmetered amount of blood is introduced onto the platform directly into assay chamber **407**, preferably, from about 15μL to about 150μL.

Fluid movement into metering capillary **402** is accompanied by filling of capillary **410**. In embodiments comprising a sacrificial valve **411** in-line with capillary **410** at a position between capillary **410** and **412** shown in Figure 6A, release of the sacrificial valve results in blood fluid flow through capillary **412** and into cell separation chamber **407**. In said embodiments, fluid flow is achieved at rotational speed f_1 with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve **411** at a position between capillary **410** and **412** shown in Figure 6A, capillary **410** preferably fills along with filling of metering capillary **402** until blood reaches capillary junction **411** at the junction between capillary **410** and capillary **412**. In such embodiments, the capillary junction has a depth of from about 0.03mm to about 2.2mm. In these embodiments, the fluid contained in metering capillary **402** is delivered into cell separation chamber **407** by rotation of the disc at a higher rotational speed f_2 of from about 250rpm to about 1500rpm (Figure 6B).

Blood flows into cell separation chamber **407** with displaced air flowing through air channel **414**, and may be vented to the surface of the disc or in communication with metering capillary **402** or entry port **401** (Figure 6C). As blood flows into cell separation chamber **407**, the fluid component of the blood wicks into matrix **406**; the pore size of the matrix is chosen to prevent the cellular components of the blood from entering the matrix. In a preferred embodiment, matrix **406** is arranged in cell separation chamber **407** so that the matrix is in contact with or more preferably adhered to the lower surface of cell separation chamber **407**. Blood fluid, such as plasma or serum, traverses matrix **406** by wicking and under rotation-induced pressure, saturating the matrix and filling a space formed between the top surface of the matrix and the top surface of cell separation chamber **407** (Figure 6C).

Blood fluid exits cell separation chamber **407** through capillary **420** and flows into assay chamber **421** (Figure 6D), with displaced air flowing through air channel **414**, and may be vented to the surface of the disc or in communication with cell separation chamber **407** (Figure 5.4). Blood

fluid flows into assay chamber **421** and wicks into matrix **422**. As the blood fluid wicks into matrix **422**, dried reagents are solubilized and the reaction of the blood component catalyzed by said reagents proceeds as described below. The timescale over which these reactions take place is chosen to be long compared with the time it takes for the fluid component of blood to saturate the matrix **422**; however, in preferred embodiments, the reaction(s) goes to completion within about 0.5 min to about 5min. Reaction of the blood component(s) with reagents produce a colored product (Figure 6E). In preferred embodiments, detection of colored product is performed spectrophotometrically, although visual inspection is also contemplated in alternative embodiments of the invention. The amount of colored product produced is detected and the amount of glucose in the blood sample determined thereby.

Another alternative embodiment of a blood glucose assay microsystems platform is shown in Figures 7A -7D. Construction of the disk embodiments of the platforms of the invention were as described above.

The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber **501**, metering capillary **502**, overflow capillary **503**, and overflow chamber **505**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **514**, and capillary junction(s) **515**, that permit venting of air displaced by fluid movement on the platform.

Metering capillary **502** is fluidly connected with capillary **510**, having a cross-sectional diameter of from about 0.02mm to about 2mm and extending from about 1mm to about 5cm from the blood fluid chamber. Capillary **510** is also fluidly connected with mixing chamber **515** that is from about 0.02mm to about 3cm deep and having a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Mixing chamber **515** is also fluidly connected with capillary **520** having a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **520** is further fluidly connected with reagent chamber **521**, wherein reagents **508** for detecting blood glucose and determining the concentration thereof are stored. Reagent chamber **521** is from about 0.02mm to about 3cm deep and having a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm

to about 14cm from the center of rotation. In certain embodiments, reagents **508** are stored on the disc in solution; in these embodiments, fluid flow through capillary **520** is preferably controlled using sacrificial valve **525**. In alternative embodiments, reagents **508** are stored in reagent chamber **521** in dry form. In these embodiments, capillary **520** can preferably comprise sacrificial valve **525** or capillary valve **525** positioned between mixing chamber **515** and reagent chamber **521**. In these embodiments, reagent chamber **521** further comprises means for a user to add an appropriate amount of a reagent diluent **530**, or the platform further comprises diluent chamber **531** fluidly connected to reagent chamber **521** by way of capillary **532**. In these embodiments, reagent diluent chamber **531** that is from about 0.02mm to about 3cm deep and having a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. In these embodiments, reagents **508** are solubilized in reagent chamber **521** immediately prior to or during use.

Mixing chamber **515** is fluidly connected by capillary channel **536** having a cross-sectional diameter of from about 0.02mm to about 2mm and extending from about 1mm to about 5cm from the mixing chamber. Capillary channel **536** is further fluidly connected with mixed fluid receiving chamber **537**. Mixed fluid chamber **537** is from about 0.02mm to about 3cm deep and having a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Alternatively, capillary **536** is further fluidly connected to second mixing chamber **540**. Second mixing chamber **540** is from about 0.02mm to about 3cm deep and having a cross-sectional diameter of from about 0.02mm to about10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Second mixing chamber is fluidly connected by a capillary channel **546** which is further connected with mixed fluid receiving chamber **537**. In these embodiments, capillary channel **546** has a cross-sectional diameter of from about 0.02mm to about 2mm and extends from about 1mm to about 5cm from the second mixing chamber.

Capillary channels **510** and **520**, and capillary channels **536** and **546**, may be offset in their connection with the mixing chamber(s). As a consequence, fluid flowing through capillary channels **536** and **546** is forced to encounter the opposite wall of mixing chambers **515** and **540** before fluid flow can proceed through further capillary channels. The fluid streams entering a small channel flow

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in a laminar fashion and therefore mix only by diffusion; the mixing chamber allows the fluids to move in a turbulent fashion and thus mix more effectively. This results in the creation of turbulence in the mixed laminar fluid stream in capillary channels 536 and 546 caused by the conjoint flow of fluid from the input capillaries without appreciable mixing. The turbulence created by the structure

5   of mixing chambers 515 and 540 is sufficient to disrupt laminar flow and cause fluid mixing in the chamber prior to continued fluid flow through capillary channel 546 and into mixed fluid receiving chamber 537.

As illustrated in Figures 7A through 7D, in the use of this platform a volume of blood is applied to metering capillary 502, either directly or using the metering components of the platform

10   described above. Fluid enters the each of the capillaries 510 and 520 and stops at capillary junction(s) or sacrificial valve(s) 525.

At a rotational speed f_t of 100 to 1000rpm, the fluids from each capillary flow past capillary junction 525 and through mixing chamber 515 (Figure 7B). Alternatively, fluid flow is activated by release of sacrificial valves 525. Fluid flow within mixing chamber 515 is turbulent, in contrast

15   to fluid flow through capillaries 510 and 520, which is primarily laminar, so that mixing occurs predominantly in mixing chamber 515. Fluid flow proceeds through channel 536 and then either through second mixing chamber 540 or directly through capillary 546 into mixed fluid receiving chamber 537 (Figure 7C).

Glucose detection reagents mixed with blood reacts in mixed fluid receiving chamber 537

20   (Figure 7C). The timescale over which these reactions take place preferably goes to completion within about 0.5 min to about 5min. Reaction of the blood component(s) with reagents 508 produce a colored product (Figure 7D). After sufficient time for the reaction to proceed, the platform is centrifuged at a speed of about 500rpm to about 3000rpm, wherein said speed pellets the cellular component of the blood, particularly the red blood cell component thereof, to the "bottom" or most

25   axially distal portion of mixed fluid receiving chamber 537. Detection of the colored product of the glucose detecting reaction is performed at a position in mixed fluid receiving chamber 537 radially more proximal to the axis of rotation than the position to which the cellular fraction has been pelleted (Figure 7D). In preferred embodiments, detection is performed spectrophotometrically,

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although visual inspection is also contemplated in alternative embodiments of the invention. The amount of colored product produced is detected and the amount of glucose in the blood sample determined thereby.

Another alternative embodiment of a blood glucose assay microsystems platform is shown in Figures 8A through 8D. Construction of the disk embodiments of the platforms of the invention were as described above.

The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber **601**, metering capillary **602**, overflow capillary **603**, and overflow chamber **605**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **634**, and capillary junction(s) **635**, that permit venting of air displaced by fluid movement on the platform.

Metering capillary **602** is fluidly connected to capillary **610** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to capillary or sacrificial valve **611**. Sacrificial or capillary valve **611** is further fluidly connected with capillary **612** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **612** is further fluidly connected to cell separation chamber **607**. Cell separation chamber **607** is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 002mm to about 3cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Where capillary **610** is connected with capillary valve **611**, said capillary valve **611** is from about 0.03mm to about 2.2mm deep and has a cross-sectional diameter of from about 0.03mm to about 2.2mm.

Cell separation chamber **607** comprises a depression in the surface of the platform having a depth of from about 0.02mm to about 3cm, most preferably comprising a circular or concave depression **613** connected to capillary **612**. Depression **613** has a depth of from about 0.02mm to about 1cm and a volume of from 15μL to about 150μL. Cell separation chamber **607** also comprises a filter **606** consisting of a porous material whose pores are sized to filter red blood cells, ranging from about 0.2μm to about 2μm. Filter **606** is in contact with and more preferably adhered to the surface of cell separation chamber **607** and most preferably extends over depression **613**. Blood

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flowing into cell separation chamber **607** is directed to depression **613** by capillary **612**. As depression **613** fills, serum is both wicked and driven by rotation-induced pressure through the filter **606** to the upper surface of cell separation chamber **607**, leaving red blood cells and other cellular components trapped beneath filter **606** in depression **613**. The volume of blood fluid at the upper

5   surface of cell separation chamber **607** is from about 15μL to about 150μL.

Cell separation chamber **607** is fluidly connected at a radial position distal from the axis of rotation to capillary **620** that is from about 0.02mm to about 2mm deep, has a cross-sectional diameter of from about 0.02mm to about 2mm and extends from about 1mm to about 5cm from cell separation chamber **607**. Capillary **620** is further fluidly connected with mixing chamber **615** that

10   is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Capillary **620** is further fluidly connected with reagent chamber **621**, wherein reagents **608** for detecting blood glucose and determining the concentration thereof is stored. Reagent chamber **621** is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to

15   about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. In certain embodiments, reagents **608** are stored on the disc in solution; in these embodiments, fluid flow through capillary **620** is preferably controlled using sacrificial valve **625**. In alternative embodiments, reagents **608** are stored in reagent chamber **621** in dry form. In these embodiments, the platform further comprises diluent chamber **631** fluidly connected to reagent chamber **621** by

20   way of capillary **632**. Diluent chamber **631** is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Capillary **632** has a cross-sectional diameter of from about 0.02mm to about 2mm, and is positioned from about 1.2cm to about 14cm from the center of rotation. In these embodiments, reagents **608** are solubilized in reagent chamber **621** immediately

25   prior to or during use.

Mixing chamber **615** is fluidly connected by capillary channel **636** having a cross-sectional diameter of from about 0.02mm to about 2mm and extending from about 1mm to about 5cm from the mixing chamber. Capillary channel **636** is further fluidly connected with mixed fluid receiving

chamber **637**. Mixed fluid chamber **637** is from about 0.02mm to about 2mm deep, has a cross-sectional diameter of from about 0.02mm to about 2mm, and is positioned from about 1.2cm to about 14cm from the center of rotation.

Alternatively, capillary **636** is further fluidly connected to second mixing chamber **640**. Second mixing chamber **640** is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 10cm from the center of rotation. Second mixing chamber is fluidly connected by a capillary channel **646** which is further connected with mixed fluid receiving chamber **637**. Capillary channel **646** has a cross-sectional diameter of from about 0.02mm to about2mm and extends from about 1mm to about 5cm from the second mixing chamber.

Capillary channels **610** and **620**, and capillary channels **636** and **646**, may be offset in their connection with the mixing chamber(s). As a consequence, fluid flowing through capillary channels **636** and **646** are forced to encounter the opposite wall of mixing chambers **615** and **640** before fluid flow can proceed through further capillary channels. This results in the creation of turbulence in the mixed laminar fluid stream in capillary channels **636** and **646** caused by the conjoint flow of fluid from the input capillaries without appreciable mixing. The turbulence created by the structure of mixing chambers **615** and **640** is sufficient to disrupt laminar flow and cause fluid mixing in the chamber prior to continued fluid flow through capillary channel **646** and into mixed fluid receiving chamber **637**. Mixed fluid receiving chamber **637** is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about1.2cm to about 14cm from the center of rotation.

As illustrated in Figures 8A through 8D, in the use of this platform a volume of blood is applied to metering capillary **602**, either directly or using the metering components of the platform described above. Blood flows into cell separation chamber **607** with displaced air flowing through air channel **654**, and may be vented to the surface of the disc or in communication with blood fluid chamber **604** (Figure 8B). As blood flows into cell separation chamber **607**, the fluid component of the blood wicks into matrix **606**; the pore size of the matrix (from about 0.2μm to about 2μm) is chosen to prevent the cellular components of the blood from entering the matrix. In a preferred

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embodiment, matrix **606** is arranged in cell separation chamber **607** so that the matrix is in contact with or more preferably adhered to the lower surface of cell separation chamber **607**. Blood fluid, such as plasma or serum, traverses matrix **606** by wicking and under rotation-induced pressure, saturating the matrix and filling a space formed between the top surface of the matrix and the top

5   surface of cell separation chamber **607** (Figure 8C).

Blood fluid exits cell separation chamber **607** through capillary **610** and flows into mixing chamber **615**. Similarly, at a rotational speed of from about 200rpm to about 2000rpm sufficient to overcome capillary valve **625**, or upon release of sacrificial valve **625**, solubilized reagents **608** flow through capillary **620** and into mixing chamber **615** (Figure 8C). Fluid flow within mixing chamber

10   **615** is turbulent, in contrast to fluid flow through capillaries **610** and **620**, which is primarily laminar, so that mixing occurs predominantly in mixing chamber **615**. Fluid flow proceeds through channel **636** and then either through second mixing chamber **640** or directly through capillary **646** into mixed fluid receiving chamber **637** (Figure 8D).
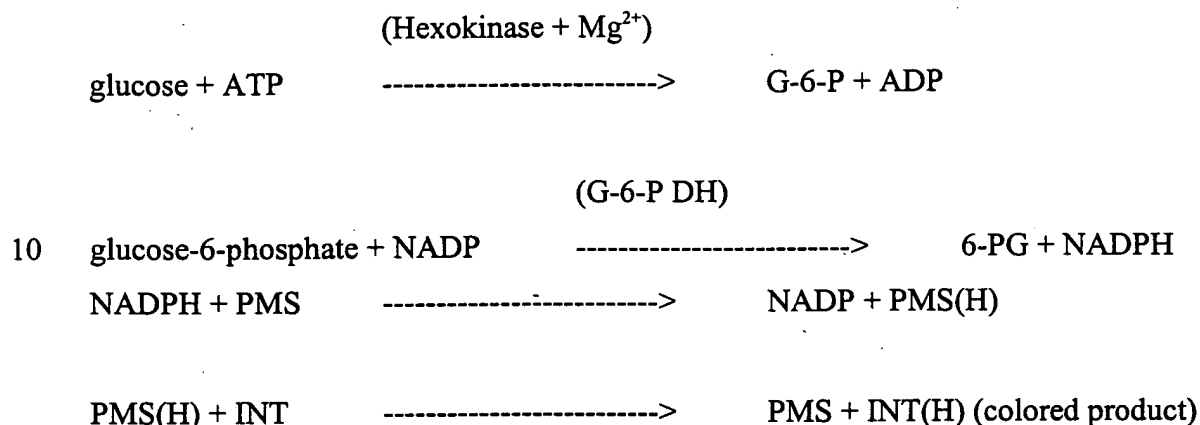
Glucose detection reagents mixed with blood in mixed fluid receiving chamber **637** (Figure

15   8D). The timescale over which these reactions take place preferably goes to completion within about 0.5 min. to about 5min. Reaction of the blood component(s) with reagents **608** produce a colored product (Figure 8D). Detection of the colored product of the glucose detecting reaction is performed in mixed fluid receiving chamber **637**. In preferred embodiments, detection is performed spectrophotometrically, although visual inspection is also contemplated in alternative embodiments

20   of the invention. The amount of colored product produced is detected and the amount of glucose in the blood sample determined thereby.

The microfluidics structures of the invention are used to detect the amount or concentration of a chemical species in a solution or complex mixture, most preferably an aqueous solution or mixture. In certain preferred embodiments, the chemical species to be detected in glucose in blood.

25   In one embodiment, blood glucose in blood is detected using a hexokinase assay.

In this assay, hexokinase converts glucose to glucose-6-phosphate (G-6-P) in the presence of magnesium cation. G-6-P is then converted to 6-phosphogluconate (6-PG) by glucose-6-phosphate dehydrogenase in the presence of NADP   producing a stoichiometric

amount of NADPH. NADPH is oxidized by reaction with phenazine methosulfate (PMS) as an intermediate, which is then oxidized by reaction with indotetrotazolium chloride, which forms a colored product that absorbs visible light at 520nm. Optimum reagent component concentrations vary according to the specifics of the application of this chemical assay, as well understood and

5   practiced by one versed in the art. This reaction scheme is illustrated as follows:

$$(\text{Hexokinase} + Mg^{2+})$$

glucose + ATP   ------------------------->   G-6-P + ADP

$$(\text{G-6-P DH})$$

10   glucose-6-phosphate + NADP   ------------------------->   6-PG + NADPH

NADPH + PMS   ------------------------->   NADP + PMS(H)

PMS(H) + INT   ------------------------->   PMS + INT(H) (colored product)

15   The amount of glucose in the blood fluid is directly related to the amount of reduced indotetrotazolium chloride, and the concentration of reduced indotetrotazolium chloride was related to glucose concentration using light spectroscopy as described below, as understood by those with skill in the art. All of these reagents are preferably provided as dried reagents 108 applied to the disc, *for example*, comprising matrix 106 shown in Figures 1 through 8. These reagents can be

20   lyophilized or air dried directly onto the surface of the disk, for example by "ink-jet" methods, or can be applied as dried beads or other particulate components.

In a variation of this reaction scheme, reduction of NADP is assayed directly without the use of PMS or indotetrazolium chloride. In this embodiment, the concentration of oxidized NADP is detected spectrophotometrically by illuminating the sample with light at 340nm, and detecting

25   absorbance. The amount of glucose in the sample is inversely proportional to the amount of oxidized NADP present in the sample after reaction. In these embodiments, the amount of NADP must be precisely controlled to be certain that the inverse proportionality between oxidized NADP and glucose is maintained.
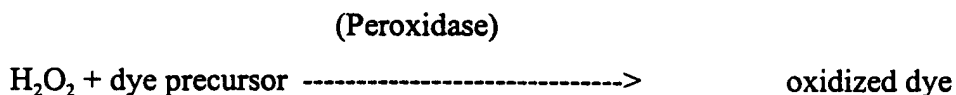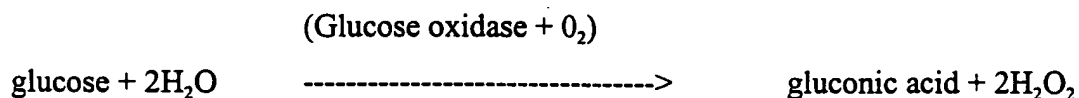
Detection is performed by transmission, reflection or reflectance spectroscopy. In transmission spectroscopy, light at a wavelength of 520nm produced by a narrow band light source, most preferably using a light emitting diode (LED) with a filter, enters one face of an assay chamber, more preferably at a position in the chamber that comprises a detection cell, and the light transmitted

5   through the detection cell is detected using a photomultiplier tube, photodiode, photodiode array, or avalanche photodiode. The photomultiplier tube is calibrated so that the amount of transmitted light detected is interpreted according to Beer's law to determine optical density and hence concentration of glucose in the sample by the well-known linear relation between the logarithm of the incident intensity/transmitted intensity and the concentration of colored (absorbing) product.

10   Alternatively, production of colored reaction products is detected by reflection spectroscopy. Light at a wavelength of 520nm is produced by a narrow band light source, most preferably a further combination with a monochromator, grating, or filter. Monochromatic light sources such as lasers or rare gas lamps as well as quasi-monochromatic light sources such as LEDs may also be used and enters one face of an assay chamber, more preferably at a position in the chamber that comprises a

15   detection cell. In preferred embodiments, the face of the assay chamber or detection cell opposite to the face illuminated by the light source comprises a reflective surface, preferably formed using a reflective material, including but not limited to an aluminum layer, a metallized glass, a mirror, and high gloss paint, or a diffusely reflecting surface such as TiO_2, underneath the colored fluid, which advantageously decreases the contribution of scratches or rotor wobble. Illuminated light is

20   reflected back through the detection cell at a direct or through an oblique angle and is detected using a photomultiplier tube photodiode, photodiode array, or avalanche photodiode.

For embodiments of the platforms of the invention wherein the assay chamber comprises a solid or porous matrix, production of colored reaction products is most preferably detected by a variation on reflection spectroscopy described above. Light at a wavelength of 520nm is produced

25   by a narrow band light source, most preferably in further combination with a monochromator is produced that enters one face of an assay chamber, more preferably at a position in the chamber that comprises a detection cell. Light is absorbed from the colored reaction products comprising the

matrix and is scattered from the matrix which comprises a diffusely-scattering material. The diffusely scattered, reflected light is detected.

In an alternative reaction protocol, glucose oxidase is used to produce hydrogen peroxide by oxidation of glucose in the blood sample. In this reaction scheme, glucose oxidase converts glucose

5   to gluconic acid and hydrogen peroxide; a twice-stoichiometric amount of hydrogen peroxide is produced relative to the amount of glucose present in the blood sample. The hydrogen peroxide then oxidizes a dye precursor present in the assay chamber or preferably within a detection cell, yielding a colored product. A variety of dye precursors are useful in the practice of this aspect of the invention, including but not limited to O-dianisidine, O-toluidine, O-tolidine, benzidine, 2,2'-

10   azinodi-(3-ethylbenzthiazoline sulfonic acid), 3-methyl-2-benzthiazolinone hydrazone plus N,N-dimethylaniline, phenyl   plus 4-aminophenzanone, sulfonated 2,4-dichlorophenol plus 4-aminophenzanone, 3-methyl-2-benzothiazolinone hydrazone plus 3-(dimethylamino) benzoic acid, 2-methoxy-4-allyl phenol and 4-aminoantipyrene-dimethylaniline. Optimum reagent component concentration vary according to the specifics of the application of this chemical assay, as well

15   understood and practiced by one versed in the art. This reaction scheme is illustrated as follows:

(Glucose oxidase + O_2)

glucose + 2H$_2$O   ------------------------------>   gluconic acid + 2H$_2$O$_2$

(Peroxidase)

20   H$_2$O$_2$ + dye precursor ----------------------------->   oxidized dye

The amount of glucose in the blood fluid is directly related to the amount of oxidized dye produced. The concentration of oxidized dye produced is determined by Beers law and light spectroscopy measurements. The total amount of oxidized dye produced is then used to calculate the sample

25   glucose concentration. Alternatively and preferably, the rate of oxidized dye production is measured and that rate related to the sample glucose concentration. Calibration, to relate glucose concentration to optical measurements calculated by either method above, are well understood by one versed in this art. All of these reagents are preferably provided as dried reagents **108** applied to the disc, *for*

example, comprising matrix **106** shown in Figures 1 through 8. These reagents can be applied, by for example methods, including but not limited to, filling, spraying, dipping, rolling and stamping a solution containing reagent components followed by lyophilization or air drying. Several applications may be made sequentially, with different components.

5   The invention also provides microsystem platforms for performing separations of particular components of a solution or complex mixture. In particular, the invention provides disc embodiments of the platforms of the invention comprising separation chambers containing components or matrices that specifically bind and retain particular chemical species comprising a chemical solution or complex mixture. This aspect of the invention is illustrated by a microfluidics

10   array for separating glycated hemoglobin from a blood sample, as shown in Figure 9.

Construction of the disk embodiments of the platforms of the invention were as described above. The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber **701**, metering capillary **702**, overflow capillary **703**, and overflow chamber **705**. As in Example 1, each

15   of the overflow and fluid chambers is also connected with air ports or air channels, such as **754**, and capillary junction(s) **755**, that permit venting of air displaced by fluid movement on the platform.

Metering capillary **702** is fluidly connected to capillary **710** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **710** is further fluidly connected to mixing chamber **715** that is from about 0.02mm to about 3cm

20   deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Lysis buffer chamber **716** containing blood lysis buffer is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Lysis buffer chamber **716** is positioned more proximally to the axis of rotation that mixing

25   chamber **715**, and has a volumetric capacity of from about 15μL to about 150μL of lysis buffer, composed of 0.1% Triton X100 in 50mM Tris pH 9.5. Lysis buffer chamber **715** is fluidly connected through capillary **718** to mixing chamber **715**.

Mixing chamber **715** is fluidly connected to capillary **717** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to secondary metering structure **719**. Secondary metering structure **719** is from about 0.02mm to about 3cm deep, and is positioned from about 1.2cm to about 14cm from the center of

5   rotation. Secondary metering structure **719** is constructed to comprise three sections. A first metering section is arranged proximal to the entry position of capillary **717** and is separated from a second metering section by a septum that extends from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produces a fluid connection between the first metering section having a volumetric capacity of from about 5μL to about 15μL

10   and second metering section having a volumetric capacity of from about 5μL to about 15μL. An overflow chamber is positioned adjacent to the second metering section and separated by a septum that extends from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produces a fluid connection between the second metering section and the overflow sections of the secondary metering structure **719**.

15   Capillary **721** is in fluid connection with secondary metering structure **719** at the distal wall of the first metering section. Capillary **721** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is fluidly connected to boronate affinity matrix chamber **722**. Boronate affinity matrix chamber **722** is from about 0.02mm to about 0.3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned

20   from about 1.2cm to about 14cm from the axis of rotation. Boronate affinity matrix chamber **722** further comprises 20-50μL boronate-functionalized agarose beads having a mean diameter of about 60μm; the beads are maintained in the chamber **722** using a porous frit **727**. Fluid flow through capillary **721** is connected to capillary or sacrificial valve **723**. Capillary **724** is in fluid connection with secondary metering structure **719** at the distal wall of the second metering section. Capillary

25   **724** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to read window **726**. Read window **726** is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation. Read window is comprised

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of a material transparent to light at a wavelength of about 430nm. Fluid flow through capillary 724 is connected to capillary or sacrificial valve 725.

Boronate affinity matrix chamber 722 is further fluidly connected to capillary 728. Capillary 728 is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to column preparation buffer reservoir 729. Column preparation buffer reservoir 729 is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 12cm from the axis of rotation, more proximal than boronate affinity matrix chamber 722. Column preparation buffer reservoir 729 comprises from about 100μL to about 500μL of column preparation buffer comprising magnesium chloride, taurine, D,L-methionine, sodium hydroxide, antibiotics and stabilizers (obtained from IsoLab as described in the Examples below). Fluid flow through capillary 728 is connected to capillary or sacrificial valve 735.

Boronate affinity matrix chamber 722 is further fluidly connected to capillary 730. Capillary 730 is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to column wash buffer reservoir 731. Column wash buffer reservoir 731 is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation, more proximal than boronate affinity matrix chamber 722. Column wash buffer reservoir 731 comprises from about 100μL to about 500μL of column preparation buffer as described above. Fluid flow through capillary 730 is connected to capillary or sacrificial valve 736.

In alternative embodiments, column preparation buffer reservoir 729 and column wash buffer reservoir 731 can be the same reservoir, or can be fluidly connected as shown in Figure 9A.

Boronate affinity matrix chamber 722 is further fluidly connected to capillary 732. Capillary 732 is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to read window 726.

Read window 726 is further fluidly connected to capillary 733. Capillary 733 is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to waste reservoir 734. Waste reservoir 734 is from about 0.02mm to about 3cm

deep and has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation.

As illustrated in Figure 9A, in the use of this platform a volume of blood from about 15µL to about 150µL is applied to metering capillary **702**, either directly or using the metering components of the platform described above. Blood flowing through capillary **710** and lysis buffer flowing through capillary **718** are mixed in mixing chamber **715** by overcoming capillary valve **711** or release of sacrificial valve **711**. A volume of lysis buffer from about 25µL to about 90µL was mixed with the blood sample. Fluid flow within mixing chamber **715** is turbulent, in contrast to fluid flow through capillaries **710** and **718**, which is primarily laminar, so that mixing occurs predominantly in mixing chamber **715**. Fluid flow proceeds through channel **717** and into secondary metering structure **719**.

The mixture of lysis buffer and blood, comprising a lysed blood sample, flows at a rotational speed f_2 from about 200rpm to about 2000rpm into secondary metering structure **719**. The lysed blood sample enters and fills the first section of secondary metering structure **719**. Continued lysed blood sample flow into secondary metering structure **719** then fills the second section of secondary metering structure **719**. Any additional lysed blood sample then empties into the overflow chamber of secondary metering structure **719**. Most preferably, a sufficient volume of lysis buffer and blood sample is applied to the disc to fill at least the first and second metered sections of secondary metering structure **719**.

After the lysed blood sample is completely transferred to secondary metering structure **719**, capillary or sacrificial valve **735** is released, allowing from 100µL to about 500µL of column preparation buffer to flow at rotational speed f_2 through capillary **730** and into boronate affinity matrix **722**. Continued or discontinuous rotation motivates column preparation buffer through boronate affinity matrix **722**, capillary **732**, read window **726**, capillary **733** and into waste reservoir **734**.

After the column preparation buffer is applied to boronate affinity matrix chamber **722**, capillary or sacrificial valve **723** is released, allowing the metered lysed blood sample from the first metered section of secondary metering structure **719** through capillary **721** and into boronate affinity

matrix **722** and allowed to incubate in the affinity matrix chamber for from about 0.5 to about 5min. Capillary or sacrificial valve **736** is then released, allowing from 100μL to about 500μL of column wash buffer to flow at rotational speed f_2 through capillary **730** and into boronate affinity matrix **722**. Continued or discontinuous rotation motivates column preparation buffer through boronate affinity

5   matrix **722**, capillary **732** and into read window **726**. During fluid flow of wash buffer through the boronate affinity matrix chamber, read window is preferably illuminated by light at a wavelength of 430nm and the concentration of hemoglobin in the sample after glycated hemoglobin has been removed by the boronate affinity matrix is determined thereby.

Capillary or sacrificial valve **725** is released at rotational speed f_3 of from about 100-

10   1000rpm and the metered lysed blood sample from the second metered section of secondary metering structure **719** flows through capillary **724** and into read window **726**. Read window is then illuminated by light at a wavelength of 430nm and the concentration of hemoglobin in the sample determined transmission, reflection, or reflectance spectroscopy. The amount of glycated hemoglobin in the sample is determined by subtracting the amount of hemoglobin obtained in the

15   first reading from the amount of hemoglobin obtained in the second reading.

An alternative embodiment of the glycated hemoglobin assay microsystem platform of the invention is shown in Figure 10. Construction of the disk embodiments of the platforms of the invention were as described above. The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising

20   sample entry port chamber **801**, metering chamber **802**, overflow capillary **803**, and overflow chamber **805**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **854**, and capillary junction(s) **855**, that permit venting of air displaced by fluid movement on the platform.

Metering capillary **802** is fluidly connected to capillary **810** that is from about 0.02mm to

25   about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **810** is further fluidly connected to mixing chamber **815** that is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Lysis buffer chamber **816** containing blood

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lysis buffer is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Lysis buffer chamber **816** is positioned more proximally to the axis of rotation that mixing chamber **815**, and has a volumetric capacity of from about 15µL to about 150µL of lysis buffer, composed of 0.1% Triton X100 in 50mM Tris pH 9.5. Lysis buffer chamber **815** is fluidly connected through capillary **818** to mixing chamber **815**.

Mixing chamber **815** is fluidly connected to capillary **817** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to secondary metering structure **819**. Secondary metering structure **819** is from about 0.02mm to about 3cm deep, and is positioned from about 1.2cm to about 14cm from the center of rotation. Secondary metering structure **819** is constructed to comprise two sections. A metering section is arranged proximal to the entry position of capillary **817** and is separated from an overflow section by a septum that extends from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produces a fluid connection between the metering section having a volumetric capacity of from about 5µL to about 15µL and the overflow chamber.

Capillary **821** is in fluid connection with secondary metering structure **819** at the distal wall of the first metering section. Capillary **821** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is fluidly connected to boronate affinity matrix chamber **822**. Boronate affinity matrix chamber **822** is from about 0.02mm to about 0.3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation. Boronate affinity matrix chamber **822** further comprises 20-50µL boronate-functionalized agarose beads having a mean diameter of about 60µm; the beads are maintained in the chamber **822** using a porous frit **827**. Boronate affinity matrix chamber **822** further comprises at least one surface that is translucent to light of at least wavelength of about 430nm, permitting direct illumination and interrogation of the amount of glycated hemoglobin that is bound thereto. Fluid flow through capillary **821** is connected to capillary or sacrificial valve **°23**.

Boronate affinity matrix chamber **822** is further fluidly connected to capillary **828**. Capillary **828** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to column preparation buffer reservoir **829**. Column preparation buffer reservoir **829** is from about 0.02mm to about 3cm deep and has a cross-sectional

5   diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 12cm from the axis of rotation, more proximal than boronate affinity matrix chamber **822**. Column preparation buffer reservoir **829** comprises from about 100μL to about 500μL of column preparation buffer comprising magnesium chloride, taurine, D,L-methionine, sodium hydroxide, antibiotics and stabilizers (obtained from IsoLab as described in the Examples below). Fluid flow through capillary

10   **828** is connected to capillary or sacrificial valve **835**.

Boronate affinity matrix chamber **822** is further fluidly connected to capillary **830**. Capillary **830** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to column wash buffer reservoir **831**. Column wash buffer reservoir **831** is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from

15   about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation, more proximal than boronate affinity matrix chamber **822**. Column wash buffer reservoir **831** comprises from about 100μL to about 500μL of column preparation buffer as described above. Fluid flow through capillary **830** is connected to capillary or sacrificial valve **836**.

In alternative embodiments, column preparation buffer reservoir **829** and column wash buffer

20   reservoir **831** can be the same reservoir, or can be fluidly connected as shown in Figure 10A.

Boronate affinity matrix chamber **822** is further fluidly connected to capillary **832**. Capillary **732** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **832** is further fluidly connected with waste reservoir **834**. Waste reservoir **834** is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from

25   about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation.

As illustrated in Figure 10A, in the use of this platform a volume of blood from about 15μL to about 150μL is applied to metering capillary **802**, either directly or using the metering

components of the platform described above. Blood flowing through capillary **810** and lysis buffer flowing through capillary **818** are mixed in mixing chamber **815** by overcoming capillary valve **811** or release of sacrificial valve **811**. A volume of lysis buffer from about 25μL to about 90μL was mixed with the blood sample. Fluid flow within mixing chamber **815** is turbulent, in contrast to fluid flow through capillaries **810** and **818**, which is primarily laminar, so that mixing occurs predominantly in mixing chamber **815**. Fluid flow proceeds through channel **817** and into secondary metering structure **819**.

The mixture of lysis buffer and blood, comprising a lysed blood sample, flows at a rotational speed f_2 from about 200rpm to about 2000rpm into secondary metering structure **819**. The lysed blood sample enters and fills the metering section of secondary metering structure **819**. Any additional lysed blood sample then empties into the overflow chamber of secondary metering structure **819**. Most preferably, a sufficient volume of lysis buffer and blood sample is applied to the disc to fill at least the metered sections of secondary metering structure **819**.

After the lysed blood sample is completely transferred to secondary metering structure **819**, capillary or sacrificial valve **835** is released, allowing from 100μL to about 500μL of column preparation buffer to flow at rotational speed f_2 through capillary **830** and into boronate affinity matrix **822**. Continued or discontinuous rotation motivates column preparation buffer through boronate affinity matrix **822**, capillary **832**, and into waste reservoir **834**.

After the column preparation buffer is applied to boronate affinity matrix chamber **822**, capillary or sacrificial valve **823** is released, allowing the metered lysed blood sample from the first metered section of secondary metering structure **819** through capillary **821** and into boronate affinity matrix **822** and allowed to incubate in the affinity matrix chamber for from about 0.5 to about 5min. Capillary or sacrificial valve **836** is then released, allowing from 100μL to about 500μL of column wash buffer to flow at rotational speed f_2 through capillary **830** and into boronate affinity matrix **822**. Continued or discontinuous rotation motivates column preparation buffer through boronate affinity matrix **822**, capillary **732** and into waste reservoir **834**. During fluid flow of wash buffer through the boronate affinity matrix chamber, the chamber is preferably illuminated by light at a wavelength

of 430nm through the translucent portion of the chamber. The concentration of glycated hemoglobin in the sample is determined thereby.

An alternative embodiment of the glycated hemoglobin assay microsystem platform of the invention is shown in Figure 11. Construction of the disk embodiments of the platforms of the invention were as described above. The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber **901**, metering capillary **902**, overflow capillary **903**, and overflow chamber **905**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **954**, and capillary junction(s) **955**, that permit venting of air displaced by fluid movement on the platform.

Metered capillary **902** is fluidly connected to capillary **910** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to sacrificial wax valve **911**. Sacrificial wax valve **911** is further fluidly connected with capillary **912** that is from about 0.03mm to about 2.2mm. Capillary **912** is further fluidly connected

5   to blood lysis chamber **915** that is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 14cm from the center of rotation, and contains from about 25µL to about 90µL of blood lysis solution (0.1% Triton-X100 in 50mM Tris, pH 9.5). Blood lysis chamber **915** is further fluidly connected with capillary **918** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of

10 from about 0.02mm to about 2mm and is connected to sacrificial wax valve **913**. Sacrificial wax valve **913** is further fluidly connected with wax recrystallization chamber **914** that is from about 0.02mm to about 2mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **918** controlled by the valve.

Capillary **918** is further fluidly connected to secondary metering structure **919**. Secondary

15 metering structure **919** is from about 0.02mm to about 3cm deep, and is positioned from about 1.2cm to about 14cm from the center of rotation. Secondary metering structure **919** is constructed to comprise three sections. A first section comprises a throwaway section sample having a volumetric capacity of from about 5µL to about 10µL because it is thought that by taking the second section a more representative would be obtained. This throwaway section is arranged proximal to the entry

20 position of capillary **918** and is separated from a metering section by a septum that extends from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produces a fluid connection between the throwaway section and the metering section. The metering section has a volumetric capacity of from about 5µL to about 10µL and is fluidly connected to an overflow section having an excess volumetric capacity of from about 15µL to about

25 150µL. The volumetric capacity of the overflow section is sufficient to accommodate the largest blood fluid volume applied to the disk.

Capillary **921** is in fluid connection with secondary metering structure **919** at the distal wall of the metering section. Capillary **921** is from about 0.02mm to about 2mm deep and has a cross-

sectional diameter of from about 0.02mm to about 2mm and is connected to boronate affinity matrix

chamber **922**. Boronate affinity matrix chamber **922** is from about 0.02mm to about 3cm deep, has

a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm

to about 14cm from the axis of rotation. Boronate affinity matrix chamber **922** further comprises

5   10-50μL of boronate-functionalized agarose beads having a mean diameter of about 60μm; the beads

are maintained in the chamber **922** using a porous frit **927**. Fluid flow through capillary **921** is

connected to sacrificial valve **923**. Sacrificial wax valve **923** is further fluidly connected with wax

recrystallization chamber **924** that is from about 0.03mm to about 2.2mm deep and has a volumetric

capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the

10   lumen of the capillary **923** controlled by the valve.

Boronate affinity matrix chamber **922** is further fluidly connected to capillary **928**. Capillary

928 is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about

0.02mm to about 2mm and is connected to column wash buffer reservoir **929**. Column wash buffer

reservoir **929** is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from

15   about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of

rotation, more proximal than boronate affinity matrix chamber **922**. Column wash buffer reservoir

929 comprises from about 100μL to about 500μL of column wash buffer as described above. Fluid

flow through capillary **928** is connected to sacrificial valve **936**. Sacrificial wax valve **936** is further

fluidly connected with wax recrystallization chamber **937** that is from about 0.03mm to about 2.2mm

20   deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and

prevent occlusion of the lumen of the capillary **928** controlled by the valve.

Boronate affinity matrix chamber **922** is further fluidly connected to capillary **932**. Capillary

932 is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about

0.02mm to about 10cm and is connected to sample collection cuvette array **934**. Sample collection

25   cuvette array **934** is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of

from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis

of rotation. Sample collection cuvette array **934** is separated into a multiplicity of individual

chambers, each separated from one another by septa that extend from the distal wall of the cuvettes

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to a position adjacent to the proximal wall of the cuvettes, so that a fluid passage **950** is maintained between each of the cuvettes. The fluid passage **950** is formed by the back (proximal wall) of the sample collection cuvette array **934** and the row of septa separating each of the sections of the sample collection cuvettes **934**. Capillary **932** is fluidly connected to sample collection cuvette array

5   **934** at a position adjacent to the proximal wall of the array and directed to the cuvette most proximal to the boronate affinity matrix chamber **922**. Alternatively, the septa can be eliminated in sample collection cuvette array **934**, wherein the sample cuvette is a single chamber.

Capillary **941** is fluidly connected to secondary metering structure **919**. Capillary **941** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm

10   to about 2mm and is connected to secondary metering structure **919** at a position between the metering section and the overflow section. Capillary **941** is further fluidly connected with total hemoglobin read chamber **942**. Total hemoglobin read chamber **942** is from about 0.02mm to about 3cm deep, is positioned from about 1.2cm to about 14cm from the center of rotation, and has a volumetric capacity of from about 50μL to about 250μL. Total hemoglobin read chamber **942** is

15   positioned radially more distal from the center of rotation than secondary metering structure **919**, and comprises a read window translucent to light having a wavelength of from about 430nm. In addition, there is no capillary or sacrificial valving controlling fluid flow in capillary **941**.

The platform also comprises control sample read cuvettes **943** and **944**, advantageously positioned in proximity to total hemoglobin read chamber **942**. Control sample read cuvettes **943**

20   and **944** are each from about 0.02mm to about 3cm deep, positioned from about 1.2cm to about 14cm from the center of rotation, and have a volumetric capacity of from about 50μL to about 200μL. Control sample read cuvettes **943** and **944** comprise a read window translucent to light having a wavelength of from about 430nm. Control sample read cuvettes **943** and **944** are not fluidly connected to any other structure on the platform and contain standards and/or calibration reagents.

25   As illustrated in Figure 11, in the use of this platform a volume of blood from about 15μL to about 150μL is applied to metering capillary **902**, either directly or using the metering components of the platform described above. Release of sacrificial valve **911** and rotation of the platform at a rotational speed f_1 of from about 50rpm to about 1000rpm motivates blood flow

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through capillary **910** and into blood lysis chamber **915**. The mixture of blood and blood lysis buffer in blood lysis chamber **915** is mixed by agitation, wherein the platform is accelerated repeatedly from about +2000rpm/sec to -2000rpm/sec (wherein "+" and "-" indicate rotation in different directions) over a time period of about 30sec to about 600sec. Release of sacrificial valve **913** and

5   rotation of the platform at a rotational speed f_2 of from about 200rpm to about 2000rpm motivates the lysed blood sample to flow through capillary **918** and into secondary metering structure **919**. Continued rotation motivates lysed blood solution to fill the metering section of secondary metering structure **919**; after filling of this section of the structure, excess lysed blood sample flows through capillary **941** and into total hemoglobin read chamber **942**. After filling of total hemoglobin read

10   chamber **942**, any excess lysed blood sample is displaced into the overflow section of secondary metering structure **919**.

Release of sacrificial valve **923** and rotation of the platform at a rotational speed f_3 of from about 200rpm to about 2000rpm motivates the metered lysed blood sample in the metering section of secondary metering structure **922** to flow through capillary **921** and into boronate affinity matrix

15   chamber **922**. After incubation of the metered lysed blood sample **956** in boronate affinity matrix chamber **922**, sacrificial valve **936** is released and the platform is rotated at a rotational speed f_4 of from about 200rpm to about 3000rpm. Column wash buffer flows from column wash buffer reservoir **929** through capillary **921** and into boronate affinity matrix chamber **922**, displacing the unbound hemoglobin fraction through capillary **932** and into sample collection cuvette array **934**.

20   Continued rotation of the platform displaces the collected sample sequentially into the separate cuvettes radially away from the position of boronate affinity matrix chamber **922** on the platform. Sample collection cuvette array **934** is then interrogated by illumination with light at a wavelength of 430nm, and the concentration of non-glycated hemoglobin in the blood sample determined. Additionally, illumination of total hemoglobin read chamber **942** with light at a wavelength at

25   415nm is performed to determine the concentration of total hemoglobin in the blood sample. The amount of glycated hemoglobin is calculated by subtracting the amount of non-glycated hemoglobin in the sample from the total hemoglobin concentration in the sample. Control sample read cuvettes **943** and **944** are used to calibrate the spectrophotometric readings.

In alternative embodiments of these microfluidics systems, the boronate affinity matrix is replaced by other substances capable of differentially binding glycated or non-glycated hemoglobin species. In a first embodiment of such an alternative, m-aminophenylboronate polyacrylic acid is used to derivatize a positively-charged nylon 66 membrane (such as Biodyne-B, Pall Biosupport

5   Division, Port Washington, N.Y.). This membrane is used in substitution for the boronate-functionalized agarose beads in the boronate affinity matrix chambers of the invention. In preferred embodiments, the boronate affinity matrix chambers are modified to contain the membrane in contact with or more preferably adhered to the platform surface within the chamber, so that one face of the membrane derivatized with m-aminophenylboronate polyacrylic acid is in contact with the

10   lysed blood sample. Binding of glycated hemoglobin to the membrane is quantitated by visible light reflectance spectroscopy at a wavelength of 415nm.

A second alternative embodiment of the glycated hemoglobin microsystems assays of the invention comprises inositol hexaphosphate. In these embodiments, inositol hexaphosphate is attached (covalently or by electrostatic interactions) to a solid support, including but not limited to

15   beads, membranes, pads, etc. The lysed blood sample is treated with sodium dithionite to convert it to the deoxy form. In the microsystems platforms of the invention, an effective amount of sodium dithionite is provided with the lysis buffer, or as a component of the secondary metering structures, most preferably as a dry powder coating on the walls of one or both of the metering sections thereof. The deoxygenated lysed blood sample is then placed in contact with the solid support comprising

20   inositol hexaphosphate, preferably comprising and in substitution for the boronate affinity matrix chamber of the glycated hemoglobin platforms of the invention. In these embodiments, the portion of hemoglobin that does not bind to the inositol phosphate-containing solid support is the glycated fraction, which can be delivered to a reads chamber, cuvette or other optically-appropriate component of the platform and the amount of glycated hemoglobin determine directly by visible

25   light reflectance spectrophotometry at a wavelength of 415nm.

The invention also provides microsystem platforms for performing a multiplicity of reactions including identification of chemical species from solutions or complex mixtures and separations of particular components of a solution or complex mixture. This aspect of the invention is illustrated

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by a microfluidics array for determining glucose concentration and separating glycated hemoglobin from a blood sample, as shown in Figures 12A through 12Q and 13A through 13D.

Construction of the disk embodiments of the platforms of the invention were as described above. Figure 13B shows a detailed description of the microfluidics components of the platform, which are described in additional detail below. Figure 13C shows the geometry of a screen printed electrical lead layer deposited on a mylar substrate. Figure 13D shows the positions of screen printed heaters activated by the electrical leads of the lead layer and screen printed on mylar. Figure 13E shows a overlay of these components in the assembled disc.

Referring to the microfluidics components of the platform shown in Figure 13B, an entry port 1 is positioned on the top surface of the disc and is open for the user to apply an unmetered sample. Entry port 1 is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 14cm from the center of rotation, and is fluidly connected to capillary channel 1A that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary channel 1A is fluidly connected to metering component 2, which comprises four sections. The first section is rectangularly-shaped and extends in a direction proximal to the axis of rotation away from its fluid connection with capillary channel 1A. About half way up this rectangular section is a lateral chamber 3, which empties into a blood glucose metering chamber 4 and an overflow chamber 5. The first section of the metering component is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 14cm from the center of rotation, and has a volumetric capacity of from about 15µL to about 150µL. The lateral chamber 3 is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Blood glucose metering chamber 4 is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 14cm from the center of rotation and has a volumetric capacity of from about 1µL to about 15µL. Overflow chamber 5 is from about 0.02mm to about 3cm deep, has a cross-sectional diameter

of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 14cm from the center of rotation and has a volumetric capacity of from about 5μL to about 50μL.

Overflow chamber **5** is fluidly connected to overflow channel **8** that is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm and extends from about 10mm to about 5cm from overflow chamber **5**. Overflow channel **5** is fluidly connected to short sample detection cuvette **9** that is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 10cm from the center of rotation and has a volumetric capacity of from about 15μL to about 150μL

Blood glucose metering chamber **4** is fluidly connected to capillary **15** that is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm and extends from about 1mmto about 5cm from blood glucose metering chamber **4**. Capillary **15** is connected to sacrificial wax valve **6**, which is further fluidly connected with wax recrystallization chamber **6A** that is from about 0.03mm to about 2.2mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **15** controlled by the valve. Capillary **15** is further fluidly connected with glucose assay chamber **11** that is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 14cm from the center of rotation and has a volumetric capacity of from about 5μL to about 50μL. Glucose assay chamber **11** comprises a depression **11A** in the surface of the platform having a depth of from about 0.02mm to about 3cm, most preferably comprising a circular or concave depression connected to capillary **15** so that blood flows into the chamber through the bottom of depression **11A**. Depression **11A** is constructed to have a volumetric capacity of from half to twice the assay volume. Blood glucose assay chamber **11** also comprises a pad or matrix **10** of a hydrophilic substance possessing a pore size of 0.2 - 2.0μm, most preferably comprising a positively-charged nylon matrix having a pore size of about 0.8μm. The upper limit on pore size of matrix **10** is chosen to inhibit or prevent blood cell entry into the matrix. The matrix is positioned in blood glucose assay chamber **11** to be in fluidic contact with depression **11A**, more preferably covering depression **11A**, and most preferably having a surface area greater than the surface area of depression **11A**. The matrix was further impregnated

with immobilized reagents **11B** which produce a detectable product proportional to the amount or concentration of glucose in a blood sample. Most preferably, the detectable product is a colored product **11C**, *i.e.*, a product absorbing light at a detectable, most preferably a visible, wavelength.

Lysis metering chamber **2** is fluidly connected to capillary **7** controlled by sacrificial valve **7A**. Sacrificial wax valve **7A** is further fluidly connected with wax recrystallization chamber **7B** that is from about 0.03mm to about 2.2mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **7** controlled by the valve. Capillary **7** is from about 0.02mm to about 2mm deep, has a cross-sectional diameter of from about 0.02mm to about 2mm, extends from about 1mm to about 5cm from lysis metering chamber **2** and is fluidly connected to blood lysis chamber **16**. Blood lysis chamber **16** is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, is positioned from about 1.2cm to about 14cm from the center of rotation, and contains from about 25μL to about 90μL of blood lysis solution (0.1% Triton-X100 in 50mM Tris, pH 9.5).

Blood lysis chamber **16** is fluidly connected at a distal aspect to capillary **17** controlled by sacrificial valve **18**. Capillary **17** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm Sacrificial wax valve **18** is further fluidly connected with wax recrystallization chamber **18A** that is from about 0.03mm to about 2.2mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **17** controlled by the valve. Capillary **17** is fluidly connected to secondary metering structure **19**. Secondary metering structure **19** is from about 0.02mm to about 3cm deep, and is positioned from about 1.2cm to about 14cm from the center of rotation. Secondary metering structure **19** is constructed to comprise three sections. A first section **20** comprises a throwaway section having a volumetric capacity of from about 5μL to about 10μL because it is thought that a more representative sample would be obtained thereby. Throwaway section **20** is arranged proximal to the entry position of capillary **17** and is separated from a metering section **21** by a septum that extends from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produces a fluid connection between throwaway section **20** and the metering section **21**. Metering section has a volumetric capacity of

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from about 5μL to about 10μL and is fluidly connected to an overflow section **24** having an excess

volumetric capacity of from about 15μL to about 150μL. The volumetric capacity of the overflow

section is sufficient to accommodate the largest blood fluid volume applied to the disk.

Capillary **25** is in fluid connection with secondary metering structure **21** at the distal wall of

5   the metering section. Capillary **25** is from about 0.02mm to about 2mm deep and has a cross-

sectional diameter of from about 0.02mm to about 2mm and is connected to boronate affinity matrix

chamber **28**. Capillary **25** is fluidly connected to sacrificial wax valve **26** that is further fluidly

connected with wax recrystallization chamber **26A**   Wax recrystallization chamber **26A** is from

about 0.03mm to about 2.2mm deep and has a volumetric capacity sufficient to sequester melted wax

10   from a released wax valve and prevent occlusion of the lumen of the capillary **25** controlled by the

valve.

Boronate affinity matrix chamber **28** is from about 0.02mm to about 3cm deep, has a cross-

sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about

14cm from the axis of rotation. Boronate affinity matrix chamber **28** further comprises boronate-

15   functionalized agarose beads having a mean diameter of about 60μm; the beads are maintained in

the chamber **28** using a porous frit **29**. Boronate affinity matrix chamber **28** is further fluidly

connected to capillary **31**. Capillary **31** is from about 0.02mm to about 2mm deep and has a cross-

sectional diameter of from about 0.02mm to about 2mm and is connected to column wash buffer

reservoir **30**. Column wash buffer reservoir **30** is from about 0.02mm to about 3cm deep and has

20   a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm

to about 14cm from the axis of rotation, more proximal than boronate affinity matrix chamber **28**.

Column wash buffer reservoir **30** comprises from about 250μL to about 350μL of column wash

buffer as described above. Fluid flow through capillary **31** is connected to sacrificial valve **32**.

Sacrificial wax valve **32** is further fluidly connected with wax recrystallization chamber **32A** that

25   is from about 0.03mm to about 2.2mm deep and has a volumetric capacity sufficient to sequester

melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **32**

controlled by the valve.

Boronate affinity matrix chamber **28** is further fluidly connected to capillary **37**. Capillary **37** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to sample collection cuvette array **12**. Sample collection cuvette array **12** is from about 0.02mm to about 3cm deep and has a cross-sectional

5 diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation. Sample collection cuvette array **12** is separated into a multiplicity of individual chambers, each separated from one another by septa that extend from the distal wall of the cuvettes to a position adjacent to the proximal wall of the cuvettes, so that a fluid passage **50** is maintained between each of the cuvettes. The fluid passage **50** is formed by the back (proximal

10 wall) of the sample collection cuvette array **12** and the row of septa separating each of the sections of the sample collection cuvettes **12**. Capillary **33** is fluidly connected to sample collection cuvette array **12** at a position adjacent to the proximal wall of the array and directed to the cuvette most proximal to the boronate affinity matrix chamber **28**. In alternative embodiments, collection cuvette array **12** can be constructed without such septa, and this structure is then just a single collection

15 chamber.

Capillary **22** is fluidly connected to secondary metering structure **19**. Capillary **22** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to secondary metering structure **19** at a position between the metering section and the overflow section. Capillary **22** is further fluidly connected with total hemoglobin read

20 chamber **23**. Total hemoglobin read chamber **23** is from about 0.02mm to about 3cm deep, is positioned from about 1.2cm to about 14cm from the center of rotation, and has a volumetric capacity of from about 5µL to about 100µL. Total hemoglobin read chamber **23** is positioned radially more distal from the center of rotation than secondary metering structure **19**, and comprises a read window translucent to light having a wavelength of from about 400nm to about 950nm. In

25 addition, there is no capillary or sacrificial valving controlling fluid flow in capillary **23**.

The platform also comprises control sample read cuvettes **13** and **14**, advantageously positioned in proximity to total hemoglobin read chamber **23**. Control sample read cuvettes **13** and **14** are each from about 0.02mm to about 3cm deep, positioned from about 1.2cm to about 14cm

from the center of rotation, and have a volumetric capacity of from about 5μL to about 100μL. Control sample read cuvettes 13 and 14 comprise a read window translucent to light having a wavelength of from about 400nm to about 950nm. Control sample read cuvettes 13 and 14 are not fluidly connected to any other structure on the platform.

5   Air displacement channels 33 and capillary junction(s) 34, that permit venting of air displaced by fluid movement on the platform, are fluidly connected to the components of the platform to permit unimpeded fluid flow.

As illustrated in Figures 12 A through 12Q, in the use of this platform a volume of blood from about 15μL to about 150μL is applied to entry port 1. Blood enters lysis subvolume 2 and

10   lateral passageway 3 under the influence of gravity and capillary forces in the absence of rotation of the platform, as shown in Figure 12A. Upon rotation of the platform at a first rotational speed f_1 of from about 50rpm to about 1000rpm, blood completely fills lysis subvolume 2 and also flows through passageway 3 and into blood glucose metering chamber 4 and overflow chamber 5, shown in Figure 12B. Blood is retained in blood glucose chamber 4 either due to capillary pressure or by

15   a sacrificial valve 6, most preferably a wax valve. Similarly, blood is retained in blood lysis subvolume 2 by a valve, most preferably a sacrificial valve 7. Excess blood flows at rotational speed f_1 through overflow channel 8 and into overflow chamber 9, shown in Figures 12C and 12D. Typical values for the first rotational speed are an acceleration of about 20 to about 60 rpm/sec to a final radial velocity of about 600rpm.

20   After blood is metered and excess blood delivered to overflow chamber 9, the rotational speed of the disc is reduced to a rotational speed f_{1a} of from about 0rpm to about 500rpm, typically to about 60rpm, to perform a blanking measurement on sample collection cuvette array 12, total hemoglobin read chamber 23 and blood glucose assay chamber 11. Measurements of blanking cuvettes 13 and 14 are also advantageously performed.

25   The disc is then accelerated to a second rotational speed f_2 of about 200rpm to about 2000rpm, greater than f_1, and typically in the range of from about 800rpm to about 1000rpm. At this speed, capillary valve 6 is overcome or sacrificial valve 6 is released, and from about 1μL to about 50μL of blood from blood glucose metering chamber 4 flows through capillary 15 and into blood

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glucose assay chamber **11**, shown in Figure 12E. Upon entering assay chamber **11**, blood fluid components are forced into absorbent matrix **10** through depression **11A**. The blood fluid is incubated in matrix **10** for a time sufficient for the reagents **10A** to produce a colored product **10B** in an amount proportional to the amount of glucose in the blood fluid sample. The disc is slowed,

5   typically to a rotational speed f_4 of from about 0rpm to about 500rpm, typically about 100rpm, for glucose data acquisition using reflectance spectrometry; data acquisition as the disc is spinning down also enables to instrument to set t=0 for the assay, based on a decrease in reflectance when the matrix **10** is wet by the blood fluid components and hence the matrix's scattering decreases. Development of colored product **10B** is shown in Figure 12G.

10   The disc is then accelerated to rotational speed f_3 of about 500rpm to about 3000rpm, typically about 1000rpm, with release of sacrificial valve **7A** and fluid flow of from about 1μL to about 50μL, typically about 5μL of blood from metered subvolume **2** through capillary **7** and into blood lysis chamber **16** containing from about 25μL to about 90μL, typically about 45μL of blood lysis buffer. This is shown in Figure 12H. The mixture of blood and blood lysis buffer in blood

15   lysis chamber **16** is mixed by agitation, wherein the platform is accelerated repeatedly from about +2000rpm/sec to -2000rpm/sec (wherein □+□ and □-□ indicate rotation in different directions), typically from about 250-500rpm/sec, over a time period of about 30 seconds to about 5min, typically 1-2min, as shown in Figures 12I and 12J.

The disc then is accelerated to a rotational speed f_5 from about 200rpm to about 2000rpm and

20   typically about 750rpm, and sacrificial valve **18** is released. Lysed blood from blood lysis chamber **16** flows through capillary **17** and into secondary metering structure **19**, as shown in Figure 12K. The lysed blood solution sequentially fills throwaway section **20**, which is used as a trap for cell debris, metering section **21** and excess lysed blood then fills overflow section **24**. Filling of metering chamber **21** is immediately followed by fluid flow through capillary **22** and filling of total

25   hemoglobin read chamber **23**. The disc is spun at rotational speed f_5 for a time sufficient to substantially completely drain blood lysis chamber **16**. The configuration of the blood fluids on the disc after this spin is shown in Figure 12L.

The disc is then accelerated to a rotational speed f, from about 200rpm to about 3000rpm and typically about 750rpm, and sacrificial valve **26** is released. A metered volume of about 1μL to about 50μL, typically about 6μL of lysed blood from metering section **21** flows through capillary **27** and into boronate affinity matrix chamber **28** (shown in Figure 12M). The lysed blood solution

5   is allowed to incubate in the chamber for a time from about 30 seconds to about 5 min, typically about 1 min, sufficient for glycated hemoglobin to bind to the matrix. This aspect of the disc is illustrated in Figure 12N.

The disc is then accelerated to a rotational speed f, from about 500rpm to about 3000rpm and typically about 1000rpm, and sacrificial valve **32** is released. A volume of about 250μL to about

10   350μL, typically about 290μL of column wash buffer as described above flows from wash buffer reservoir **30** though capillary **31** and into boronate affinity matrix chamber **28** (shown in Figure 12O). The wash buffer displaces the non-glycated hemoglobin and other components of the lysed blood fluid from the affinity column matrix and into sample collection cuvette array **12**. Figures 12P through 12Q show sequential filling of the individual cuvettes in sample collection cuvette array **12**.

15   The rotation speed of the disc is reduced, to from about 0rpm to about 500rpm and typically to about 60rpm for sample collection cuvette array **12** and total hemoglobin read chamber **23** to be interrogated spectrophotometrically. The glycated fraction of the blood sample is determine algorithmically by subtracting the non-glycated hemoglobin fraction in sample collection cuvette array **12** from the total hemoglobin detected in total hemoglobin read chamber **23**.

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2.   Resistive Heater and Temperature Sensing Components

Temperature control elements are provided to control the temperature of the platform. The invention provides heating elements, specifically resistive heating elements, and elements for detecting temperature at specific positions on the platform. Heating devices are preferably arrayed

25   to control the temperature of the platform over a particular and defined area, and are provided having a steep temperature gradient with distance on the platform from the heater.

Certain resistors, including commercially-available resistive inks (available from Dupont) exhibit a positive temperature coefficient (PTC), *i.e.*, an increase in resistance with increasing

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temperature. Applying a fixed voltage across a PTC resistor screen-printed on a plastic substrate results in rapid heating, followed by self-regulation at an elevated temperature defined by the circuit design heat sink and ambient temperature. In such screen-printed resistors, connection to a power source is made by first printing parallel silver conductors followed by printing the PTC ink between

5   the conductors.

A resistive heating element comprises a conductive ink connected with electrical contacts for activation of the heater, and resistive inks applied between the conductive ink and in electrical contact therewith, wherein application of a voltage (direct or alternating current) between the conductive inks results in current flow through the resistive inks and production of heat. There are

10   two important types of resistive inks used in the resistive heating elements of this invention The first is a standard polymer thick film ink, such as Dupont 7082 or Dupont 7102 ink. These inks produce a surface temperature that is not self-limiting, and the temperature resulting from the use of these inks is dependent primarily on the magnitude of the applied voltage. In contrast, the positive temperature coefficient (PTC) inks show increase resistivity with increasing voltage, so that

15   surface temperature is self-limiting because the amount of heat-producing current goes down as the applied voltage goes up. PTC inks are characterized as having a particular temperature where this self-limiting property is first exhibited; at voltages that produce temperatures less than the critical temperature, the amount of heat is dependent on the magnitude of the applied voltage.

Resistive inks useful according to the invention include Dupont 7082, 7102, 7271, 7278 and

20   7285, and other equivalent commercially available polymer thick film ink and PTC inks.

Conductive inks useful according to the invention include Dupont 5028, 5025, Acheson 423SS, 426SS and SS24890, and other equivalent commercially available conductive inks.

Additional components of the dielectric layer that serves to insulate the electrical circuit. Dielectric layers advantageously comprise dielectric inks such as Dupont 5018A. Insulation can also

25   be achieved using pressure sensitive transfer adhesive such as 7952MP (3M Co.), or a pressure sensitive transfer adhesive deposited onto a polyester carrier layer such as 7953MP (3M Co.) or thermoplastic bonding films such as 3M 406, 560 or 615.

Resistive heaters of the invention are advantageously used to incubate fluids at a stable temperature and for melting sacrificial valves as described below, and also for thermal cyclic.

Resistive and conductive inks are preferably screen-printed using methods and techniques well known in the art. *See* Gilleo, 1995, *Polymer Thick Film* (Van Nostrand Reinhold). Inks are typically screen printed to a thickness of about 10 microns; however, repetitive screen printing of resistive inks can be used to deposit thicker layers having reduced resistances. Both conductive and resistive inks are heat cured, typically at between 110°C and 120°C for about 10 minutes. The outline of this printing process is shown in Figure 30. Importantly, each of the layers must be correctly registered with one another for resistive heating to be provided. Heaters can be screen printed to any required size; a minimum area for a screen-printed heater has been determined to be about 0.25mm^2 (0.5mm x 0.5mm).

The ability to tailor the resistance (and hence the temperature profile) of the resistive heaters using choice of ink formulation and reprinting of heater circuits provides control of the final electrical and thermal properties of the resistive heating elements of the invention. The resistance can also be controlled through connection of series and parallel configurations of resistive elements.

3.   Sacrificial Valves

The ability to specifically generate heat at a particular location on a microsystems platform of the invention also enables the use of sacrificial valves that can be released or dissolved using heat. For the purposes of this invention, the term ⬜sacrificial valve⬜ is intended to encompass materials comprising waxes, plastics, and other material that can form a solid or semi-solid fluid-tight obstruction in a microchannel, capillary, chamber, reservoir or other microfluidics component of the platforms of the invention, and that can be melted or deformed to remove the obstruction with the application of heat. Sacrificial valves are preferably made of a fungible material that can be removed from the fluid flow path. In preferred embodiments, said sacrificial valves are wax valves and are removed from the fluid flow path by heating, using any of a variety of heating means including infrared illumination and most preferably by activation of resistive heating elements on or embedded in the platform surface as described herein. For the purposes of this invention, the term ⬜wax⬜ is

intended to encompass any solid, semi-solid or viscous liquid hydrocarbon, or a plastic. Examples include mondisperse hydrocarbons such as eicosane, tetracosane and octasone, and polydisperse hydrocarbons such as paraffin. In the use of wax sacrificial valves, application of a temperature higher than the melting temperature of the wax melts the valve and removes the occlusion from the

5   microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Particularly when the sacrificial valve is melted on a rotating microsystems platform of the invention, the melted wax to flow through the microchannel, capillary or other fluidic component of the microsystems platforms of the invention and away from the original site of the valve.

One drawback, however, is the possibility that the wax will recrystallize as it flows away

10   from the original valve site, and concomitantly, away from the localized heat source. Recrystallization results in re-occlusion of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention, potentially and most likely at a site other than the site of a localized heat source, and therefore likely to foul fluid movement on the disc. One solution for this problem is the inclusion in the sacrificial wax valves of the invention of a wax

15   recrystallization chamber positioned ⬚downstream⬚ from the position of the wax valve. Preferably, the wax recrystallization chamber is fluidly connected with the microchannel, capillary or other fluidic component of the microsystems platforms of the invention that was occluded by the wax sacrificial valve. Typically, the wax recrystallization chamber is a widening of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention so that

20   recrystallized wax can harden on the walls of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention with enough distance between said walls that the recrystallized wax does not re-occlude the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Preferably, the heating element, most preferably the resistive heating element of the invention, extends past the site of the wax valve and overlaps at least

25   a portion of the wax recrystallization chamber, thereby retarding the propensity of the wax valve to recrystallize.

It is also recognized that this propensity of wax valves to recrystallize can be exploited to create a wax valve at a particular location in a microchannel, capillary or other fluidic component

of the microsystems platforms of the invention. In this embodiment, a particular location can be kept below a threshold temperature by failing to apply heat at that location, and a wax valve material can be mobilized from a storage area on a platform by heating and them allowed to flow under centripetal acceleration to a particularly ▯cold▯ site where a wax valve is desired. An advantage of

5   wax valves in this regard is that the proper positioning an activation of resistive heater elements enables flexibility in choosing when and whether a particular microchannel, capillary or other fluidic component of the microsystems platforms of the invention is to be occluded by a wax sacrificial valve.

In particularly preferred embodiments, the sacrificial valves of the invention comprise a

10   cross-linked polymer that displays thermal recover, most preferably a cross-linked, prestressed, semicrystalline polymer; an example of a commercially available embodiment of such a polymer is heat recoverable tubing (#FP301H, 3M Co., Minneapolis, MN). Using these materials, at a temperature less than the ▯melting▯ temperature (T_m), the polymer occludes a microchannel, capillary or other fluidic component of the microsystems platforms of the invention. At a

15   temperature greater than T_m, however, the polymer reverts to its pre-stressed dimensions by shrinking. Such shrinking is accompanied by release of the occlusion from the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Such embodiments are particular preferred because the polymer remains *in situ* and does not recrystallize or otherwise re-occlude the microchannel, capillary or other fluidic component of the microsystems

20   platforms of the invention. Also, such embodiments do not require the more extensive manipulation in preparing the platforms of the invention that wax valves require.

In another embodiment, the sacrificial valves of the invention comprise a thin polymeric layer or barrier dividing two liquid-containing microchannel, capillary or other fluidic component of the microsystems platforms of the invention, that can burst when sufficient temperature and/ or

25   pressure is applied.

Another embodiment of the sacrificial valves of the invention are provided wherein a screen-printed resistive heater element is itself a valve. In this embodiment, the resistive heater element is screen-printed on a substrate such as polyester that divides two liquid-containing microchannel,

capillary or other fluidic component of the microsystems platforms of the invention. In these embodiments, localized application of heat using a resistive heating element is used to melt the substrate dividing the liquid-containing microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Preferably, in this embodiment the two liquid-containing

5   microchannel, capillary or other fluidic component of the microsystems platforms of the invention are positioned in adjacent layers through the vertical thickness of the platform.

As described above, the screen-printed resistive heater elements of this invention provide localized application of heat to a microsystems platform. The degree of localization achieved using these resistive heating elements is sufficient to provide for the placement of two adjacent sacrificial

10   valves separated by a distance of 0.15cm.

4.   Detectors and Sensors

Detection systems for use on the microsystem platforms of the invention include spectroscopic, electrochemical, physical, light scattering, radioactive, and mass spectroscopic

15   detectors. Spectroscopic methods using these detectors encompass electronic spectroscopy (ultraviolet and visible light absorbance, fluorescence, luminescence, and refractive index), vibrational spectroscopy (IR and Raman), and x-ray spectroscopies (x-ray fluorescence and conventional x-ray analysis using micromachined field emitters, such as those developed by the NASA Jet Propulsion Lab, Pasadena, CA).

20   General classes of detection and representative examples of each for use with the microsystem platforms of the invention are described below. In addition, the detection implementation systems utilizing the detectors of the invention can be external to the platform, adjacent to it or integral to the disk platform.

25   Spectroscopic Methods:

1.   Fluorescence

Fluorescence detector systems developed for macroscopic uses are known in the prior art and are adapted for use with the microsystem platforms of this invention. For example, an

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excitation source such as a laser is focused on an optically-transparent section of the disk. Light from any analytically-useful portion of the electromagnetic spectrum can be coupled with a disk material that is specifically transparent to light of a particular wavelength, permitting spectral properties of the light to be determined by the product or reagent occupying the reservoir

5   interrogated by illumination with light. Alternatively, the selection of light at a particular wavelength can be paired with a material having geometries and refractive index properties resulting in total internal reflection of the illuminating light. This enables either detection of material on the surface of the disk through evanescent light propagation, or multiple reflections through the sample itself, which increases the path length considerably.

10   Alternative configurations appropriate for evanescent wave systems are provided as understood in the art (see Glass et al., 1987, Appl. Optics 26: 2181-2187). Fluorescence is coupled back into a waveguide on the disk, thereby increasing the efficiency of detection. In these embodiments, the optical component preceding the detector can include a dispersive element to permit spectral resolution. Fluorescence excitation can also be increased through

15   multiple reflections from surfaces in the device whenever noise does not scale with path length in the same way as with signal.

                In another type of fluorescence detection configuration, light of both the fluorescence excitation wavelength and the emitted light wavelength are guided through one face of the device. An angle of 90 degrees is used to separate the excitation and collection optical trains. It

20   is also possible to use other angles, including 0 degrees, whereby the excitation and emitted light travels colinearly. As long as the source light can be distinguished from the fluorescence signal, any optical geometry can be used. Optical windows suitable for spectroscopic measurement and transparent to the wavelengths used are included at appropriate positions (i.e., in "read" reservoir embodiments of detecting chambers) on the disk. The use of this type of fluorescence in

25   macroscopic systems has been disclosed by Haab et al. (1995, Anal. Chem. 67: 3253-3260).

        2.   Absorbance Detection

        Absorbance measurements can be used to detect any analyte that changes the intensity of

transmitted light by specifically absorbing energy (direct absorbance) or by changing the absorbance of another component in the system (indirect absorbance). Optical path geometry is designed to ensure that the absorbance detector is focused on a light path receiving the maximum amount of transmitted light from the illuminated sample. Both the light source and the detector

5   can be positioned external to the disk, adjacent to the disk and moved in synchrony with it, or integral to the disk itself. The sample chamber on the disk can constitute a cuvette that is illuminated and transmitted light detected in a single pass or in multiple passes, particularly when used with a stroboscopic light signal that illuminates the detection chamber t a frequency equal to the frequency of rotation or multiples thereof. Alternatively, the sample chamber can be a planar

10   waveguide, wherein the analyte interacts on the face of the waveguide and light absorbance is the result of attenuated total internal reflection (*i.e.*, the analyte reduces the intensity source light if the analyte is sequestered at the surface of the sample chamber, using, for example, specific binding to a compound embedded or attached to the chamber surface; *see* Dessy, 1989, *Anal. Chem.* 61: 2191).

15   Indirect absorbance can be used with the same optical design. For indirect absorbance measurements, the analyte does not absorb the source light; instead, a drop in absorbance of a secondary material is measured as the analyte displaces it in the sample chamber. Increased transmittance therefore corresponds to analyte concentration.

20   3.   Light scattering

Turbidity can also be measured on the disk. Optics are configured as with absorbance measurements. In this analysis, the intensity of the transmitted light is related to the concentration of the light-scattered particles in a sample. An example of an application of this type of detection method is a particle agglutination assay. Larger particles sediment in a rotating

25   disk more rapidly than smaller particles, and the turbidity of a solution in the sample chamber before and after spinning the disk can be related to the size of the particles in the chamber. If small particles are induced to aggregate only in the presence of an analyte, then turbidity measurements can be used to specifically detect the presence of an analyte in the sample

chamber. For example, small particles can be coated with an antibody to an analyte, resulting in aggregation of the particles in the presence of the analyte as antibody from more than one particle bind to the analyte. When the disk is spun after this interaction occurs, sample chambers containing analyte will be less turbid that sample chambers not containing analyte. This system

5   can be calibrated with standard amounts of analyte to provide a gauge of analyte concentration related to the turbidity of the sample under a set of standardized conditions.

Other types of light scattering detection methods are provided for use with the microsystems platforms and devices of the invention. Monochromatic light from a light source, advantageously a laser light source, is directed across the cross-sectional area of a flow channel

10   on the disk. Light scattered by particles in a sample, such as cells, is collected at several angles over the illuminated portion of the channel (*see* Rosenzweig *et al.*, 1994, *Anal. Chem.* 66: 1771-1776). Data reduction is optimally programmed directly into the device based on standards such as appropriately-sized beads to relate the signal into interpretable results. Using a calibrated set of such beads, fine discrimination between particles of different sizes can be obtained. Another

15   application for this system is flow cytometry, cell counting, cell sorting and cellular biological analysis and testing, including chemotherapeutic sensitivity and toxicology.

Analytic Methods

It will be understood that the interpretation of the optical detection data from performance

20   of analytical assays as provided by the invention may require transformation of "raw" data into information useful for the operator. For example, "reflectance" is determined as the difference between the "signal" and the "dark signal" (*i.e.*, the signal detected in the absence of transmitted light), normalized by the difference between the "reference" and the "dark reference," wherein each of the reference values is determined for a "blank" cuvette not containing a sample.

25   Similarly, the detection methods used by the invention also include methods for eliminating artifactual signal produced by interfering absorbing or light-scattering components, such as scratches or particulate matter. Advantageously, normalized readings are collected at a wavelength greater than 650nm, more preferably 660 ±10nm as a reference. This reading is then

subtracted from the sample reading to as a correction thereof.

Absorbance from certain components of biological fluid sample, such as hemoglobin in blood, are advantageously removed by using a light source with a wavelength at the absorbance peak of the interfering material; for hemoglobin, this is about 425nm. In preferred

5   embodiments, light of this wavelength is provided by a blue light-emitting diode (LED) having a wavelength of about 430nm. The light emission profile of the LED was found to overlap sufficiently with the hemoglobin absorbance profile to effectively quench the hemoglobin absorbance signal in the glucose assays of the invention. The glucose signal was then further treated analytically by subtracting a factor times the (blue-red) signal from the (orange-red)

10   signal, wherein the (orange) signal was the wavelength specific for the glucose assay product. The factor used is dependent on the optics, the reading chamber structure, spectral properties of the light sources and filters, and the spectral absorbance characteristics of hemoglobin and the colored product of the glucose oxidase reaction. The factor can be determined empirically using solutions of known glucose and hemoglobin concentrations, both singly and in combination.

15   It will be understood in the art that similar combinations of light sources and interrogated wavelengths can be advantageously used to reduce or eliminate the contribution of other interfering species in optical detection methods used according to the invention.

20   5.   Chemistries

As described above with regard to the microfluidic components of the microsystems platforms of the invention, the present invention provides platforms for performing chemical, biochemical, enzymatic, immunological and other assays on fluid samples, most preferably wherein the fluid sample is a biological fluid sample.

25   Two exemplary types of assay formats are explicitly set forth herein; one of ordinary skill will recognize that the disclosure is generally applicable to a variety of assay systems as set forth, for example, in CLINICAL GUIDE TO LABORATORY TESTS, Tietz, ed., W.B. Sanders Co: Philadelphia, 1995. A representative and non-limiting sample of assays advantageously

performed using the microsystems platforms of the invention are set forth in Table I below.

TABLE I

Assay for:	Analyte/Detection Type	Components on solid phase:
Acid Phosphatase	enzyme/colorimetric	Alpha-naphthol phosphate, Fast Red TR dye[1]
Alanine Aminotransferase	enzyme/colorimetric	Alanine, alpha-ketoglutarate, 2,4-dinitrophenylhadrazine dye
Albumin	protein/colorimetric	Bromcresol green dye
Alkaline Phosphatase	enzyme/colorimetric	p-nitrophenyl phosphate
Amylase	enzyme/colorimetric	4,6-ethylidine(G7)-p-nitrophenyl(G1)-alpha,D-maltoheptaside, alpha-glucosidase
Apolipoprotein A-1	lipoprotein/immunoturbidimetric	Anti-ApoA1 antibody, polyethylene glycol[2]
Direct Bilirubin	organic compound/colorimetric	Diazotized sulfanilic acid
Total Bilirubin	organic compound/colorimetric	Caffeine, benzoate, acetate, diazotized sulfanilic acid
Calcium	mineral/colorimetric	Cresolphthalein complexone
Cholesterol	lipid/colorimetric	Cholesterol esterase, cholesterol oxidase, 4-aminoantipyrine, p-hydroxybenzene sulfonate
Fructosamine	glycated serum protein/colorimetric	Nitroblue tetrazolium
Gamma-glutamyl transferase	enzyme/colorimetric	L-gamma-glutamyl-3-carboxy-4-nitroanilide, glycylglycine
Iron	mineral/colorimetric	Ferrozine, reducing agent
Microprotein (urine or CSF)	protein/colorimetric	Pyrogallol red-molybdate complex
Urea	organic compound/colorimetric	Diacetylmonoxime, heat
Specific Protein species, subspecies or variant	immunologically reactive proteins/immunoturbidimetry	Antibody reactive with unique species, etc, site; precipitation enhancers (e.g., anti-alpha-1-antitrypsin IgG, polyethylene glycol)[3]
Antibodies against	immunoreaction to bacteria or	Immunoreactive component from infective

1 Saw, D., et. al, *Clin. Biochem.*, 1990; **23**:505.

2 Rifai, N., Warnick, G.R., eds., Methods for Clinical Laboratory Measurement of Lipid and Lipoprotein Risk Factors, Washington, D.C.: AACC Press, 1990

3 Hills, L.P., and Tiffany, T.O., Comparison of turbidimetric and light-scattering measurements of immunoglobulins by use of a centrifugal analyzer with absorbance and fluorescence/light scattering optics. *Clin.Chem.* 1980; **26**:1466.

infectious agents	virus infection/heterogeneous enzyme immunoassay	agent, enzyme linked to antibody against the primary immunoglobulin species of reaction to the agent, enzyme substrate linked to color generation (*e.g.*, Epstein-Barr early antigen, anti-human IgG conjugated to horseradish peroxidase, 3-3'-5-5'-tetramethylbenzidine)
Drugs	immunologically reactive therapeutic drugs or drugs of abuse/homogeneous enzyme immunoassay	Competition between drug and drug-enzyme reagent for anti-drug antibody binding sites where antibody binding inhibits enzyme activity, enzyme substrate linked to color generation (*e.g.*, phenobarbital-glucose-6-phosphatase, anti-phenobarbital IgG, NAD)[4]

The requirements for performing such assays on the solid phase (such as the matrices disclosed herein) include: the ability to link, by one or a series of chemical reaction steps, the presence of

5   an analyte of interest in a fluid sample, most preferably a biological fluid sample, to the quantitative generation of a product detectable by optical methods as disclosed herein; stabilization of the necessary reagents (chemicals or biochemicals) onto the solid phase so that the reagents retain their potencies or activities.

A second general scheme for performing assays on the microsystems platforms of the

10   invention involve miniaturized versions of affinity chromatography column separations, wherein the analyte specifically binds to a material in a chamber or on a surface, most preferably a derivatized surface, of the platform, or is bound to a material such as a bead, chromatography resin, or membrane on the surface of the platform, so that the remainder of the fluid sample can be washed from the affinity matrix and the analyte separated thereby. In certain preferred

15   embodiments, the analyte is detected indirectly, wherein the biological fluid sample is interrogated after passage of the sample over the chromatograohy matrix. Such detection methods can be subtractive, wherein the interrogated optical property of the biological fluid sample after passage over the chromatography matrix is compared with the same property of a

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4 Oellerich, M.: Enzyme immunoassays in clinical chemistry: Present status and trends. *J. Clin. Chem. Clin. Biochem.* 1980; **18**: 197.

portion of the sample that has not been passed over the matrix; or directly, wherein the analyte is dissociated from the chromatography matrix (either non-specifically, using *for example* a salt or dielectric gradient, or specifically, using a binding competitor that displaces the analyte from the chromatography matrix).

5   Examples of analytes advantageously separated from biological fluid samples and the column affinity material(s) used therefore are set forth in Table II.

TABLE II

Column Affinity Material	Bound Species
Diatomateous earth	DNA
Protein A-agarose	IgG
Heparin-agarose	coagulation proteins, Protein C, growth factors, lipoproteins, steroid receptors
Blue agarose	albumin, coagulation factors, interferon, enzymes requiring cofactors with adenyl group
Streptavidin-agarose	biotinylated molecules
Con A-agarose	glycoproteins, polysaccharides with terminal mannose or glucose
Lentil lectin-agarose	glycoproteins, polysaccharides with branched mannose with fucose linked $\alpha(1,6)$ to N-acetyl-glucosamine
Wheat gern lectin-agarose	glycoproteins, polysaccharides with chitobiose core of N-linked oligosaccharides
Peanut lectin-agarose	glycoproteins, polysaccharides with terminal β-galactose
Arginine-agarose	serine proteases
Calmodulin-agarose	ATPases, phosphodiesterases, neurotransmitters, protein kinases
Gelatin-agarose	Fibronectin
Glutathione-agarose	S-transferases, glutathione-dependent proteins
Lysine-agarose	plasminogen, plasminogen activator, ribosomal RNA
DNA (denatured)-agarose	DNA polymerase, RNA polymerase, T4 polynucleotide kinase, exonuclease, deoxyribonucleases
DNA (native)-cellulose	Glucocorticoid receptor, DNA polymerase, DNA binding proteins
2'5' ADP-agarose	NADP-dependent dehydrogenases
5' AMP-agarose	NAD-dependent dehydrogenases, ATP-dependent kinases

7-Methyl-GTP-agarose	eukaryotic mRNA, cap-binding protein
Poly(U)-agarose	mRNA, reverse transcriptase, interferon, plant nucleic acids
C8-silica	Proteins

It will be appreciated that preparative embodiments of the affinity chromatographic column separations are within the scope of the invention.

The following Examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature.

EXAMPLE 1

Blood Glucose Assay Fluidics Structure

A microsystems platform provided by the invention and specifically designed for performing blood glucose assay is illustrated in Figure 1. Disk embodiments of the platforms of the invention were fashioned from machined acrylic. The overall disc dimensions include an outer radius of about 6cm and an inner radius of about 0.75cm, wherein the disk was mounted on the spindle of a rotary device. The thickness of the fluidics disc was 3.2mm, which was mounted on a conventional compact disc (CD) having a thickness of 1.2mm. All surfaces coming into contact with blood on the platform are advantageously treated with heparin, EDTA or other anticoagulants to facilitate fluid flow thereupon.

The components of the blood glucose assay were prepared as follows. Blood sample entry port chamber 101 having a depth in the platform surface of about 0.32cm and lateral dimensions of about 1cm was constructed on the platform, and designed to accommodate a volume of 100 µL. This entry port was fluidly connected with a metering capillary 102 having a square cross-sectional diameter of about 0.1cm deep x 0.5cm wideand proximal ends rounded with respect to entry port 101; the length of this metering capillary array was sufficient to contain a total volume of about 16µL. The entry port was also fluidly connected with an overflow capillary 103 having a cross-sectional diameter of about 0.05 cm x 0.075cm and proximal ends rounded with respect to entry port

101. The overflow capillary was fluidly connected with a two-layered overflow chamber **105** having a first depth in the platform of about 0.025cm and a second depth in the platform of about 0.25cm, greater than the depth of the overflow capillary **103**. Metering capillary **102** was fluidly connected to metered blood fluid chamber **104** having a depth in the platform surface of 1mm and

5   greater than the depth of the metering capillary **102**. Each of the overflow and fluid chambers was also connected with air ports or air channels, such as **114**, that have dimensions of 0.025cm deep and permitted venting of air displaced by fluid movement on the platform. A capillary junction **115** that was .051cm deep was present in the air channel to prevent fluid flow into the air channel.

Entry port **101** was positioned on the platform about 2cm from the center of rotation.

10   Metering chamber **102** extended about 1cm from entry port **101**. The extent of the length of overflow capillary **103** was 300% greater than the extent of the length of metering capillary **102**. The position of blood fluid chamber **104** was about 4.6cm from the center of rotation, and the position of overflow chamber **105** was about 4cm from the axis of rotation.

Blood fluid chamber **104** acted as a capillary barrier that prevented fluid flow from metering

15   chamber **102** at a first, non-zero rotational speed f_1 of about 300rpm that was sufficient to permit fluid flow comprising overflow from the entry port **101** through overflow capillary **103** and into overflow chamber **105**. This capillary boundary was constructed to be overcome at a second rotational speed f_2 of about 600rpm (so that $f_2 > f_1$). Blood fluid chamber **104** was fluidly connected to capillary **110** that was about 0.025cm deep and had a cross-sectional diameter of about 0.025cm

20   and was connected to capillary or sacrificial valve **111**. Sacrificial valve **111** was further fluidly connected with capillary **112** that was about 0.25mm deep and had a cross-sectional diameter of about 0.05cm, and capillary **112** was fluidly connected to assay chamber **107**. Sacrificial valve chamber **111** was positioned to sequester melted wax produced by release of the sacrificial valve.

Assay chamber **107** comprised a depression in the surface of the platform having a depth of

25   about 0.1cm, and further comprised a circular or rectangular concave depression **113** connected to capillary **112**. Assay chamber **107** also comprised a pad or matrix **106** comprising a positively-charged nylon matrix having a pore size of about $0.8\mu m$. The pore size of matrix **106** was chosen to inhibit or prevent blood cell entry into the matrix. The matrix was positioned in assay chamber

107 to be in fluidic contact with depression 113, covering depression 113 and having a surface area greater than the surface area of depression 113. The matrix was impregnated with immobilized reagents 108 which produce a detectable product proportional to the concentration of glucose in a blood sample. The detectable product was a colored product 109, *i.e.*, a product absorbing light at a detectable, visible wavelength.

As illustrated in Figures 2A through 2E, in the use of this platform an imprecise volume (about 30µL of fluid) of blood was applied to the entry port 101. The fluid wicked into air channel 114 and was stopped by capillary junction 115. Fluid also wicked into metering capillary 102 and overflow capillary 103. Fluid flowed through the metering capillary 102 and overflow capillary 103 at no rotational speed until the fluid reached capillary junctions at the junction between metering chamber 102 and blood fluid chamber 104 and overflow capillary 103 and overflow chamber 105. Metering capillary 102 was constructed to define a precise volume of about 15µL of blood between entry port 101 and the capillary junction at fluid chamber 104, which was designed to be at least the amount of the fluid placed by the user in entry port 101.

After sample loading by a user and filling of metering chamber 102 and overflow capillary 103 at zero rotational speed, the platform was spun at a first rotational speed f_1 of about 300rpm, which was sufficient to motivate fluid flow through the overflow capillary 103 in this microfluidics array, wherein entry port 101 had a depth of about 0.3cm, metering chamber 102 had dimensions of about 0.1cm deep x 0..4cm wide in cross-section and about 0.5cm in length from the center of rotation, and overflow capillary 103 had dimensions of about 0.05cm x 0.075cm in cross-section and about 2.7cm in length from the center of rotation.

Due to the greater distance from the center of rotation of the end of overflow capillary 103 than the end of metering chamber 102, at rotational speed f_1 fluid flowed through overflow capillary 103 into overflow chamber 105. The platform was spun until all excess fluid was evacuated from entry port 101 and into overflow chamber 105, except the fluid contained in metering capillary 102.

At a second rotational speed f_2 of about 1000rpm, the precise amount of fluid (16µL) contained in metering capillary 102 was delivered into fluid chamber 104. Fluid movement into fluid chamber 104 was accompanied by filling of capillary 110.

In embodiments comprising a sacrificial valve 111 in-line with capillary 110 at a position between capillary 110 and 112 shown in Figure 2A, release of the sacrificial valve resulted in fluid flow through capillary 112 and into assay chamber 107. In said embodiments, fluid flow was achieved at rotational speed f_2 with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve 111 at a position between capillary 110 and 112 shown in Figure 2B, capillary 110 filled along with filling of blood fluid chamber 104 until blood reached capillary junction 111 at the junction between capillary 110 and capillary 112; in such embodiments, the capillary junction had a depth of about 0.05cm. At a third rotational speed f_3 of about 500rpm, the fluid contained in blood fluid chamber 104 was delivered into assay chamber 107 (Figure 2B). Blood flowing into assay chamber 107 was preferentially directed to depression 113 in the assay chamber; the dimensions of depression 113 are conveniently chosen to be able to contain substantially all of the blood fluid of the sample metered through metering chamber 102 into assay chamber 107 (Figure 2C). Displaced air flows through air channel 114, and was vented to the surface of the disc.

As blood flowed into depression 113, the fluid component of the blood is driven by pressure and hydrophilic forces into matrix 106, comprising a positively-charged nylon matrix having a pore size of about 0.8μm; this pore size was chosen to prevent the cellular components of the blood from entering the matrix (Figure 2D). The cellular blood components were retained in depression 113 and the fluid component was efficiently distributed into matrix 106. As the fluid component of the blood entered matrix 106, dried reagents 108 were solubilized and the reaction of the blood component catalyzed by said reagents proceeded. This reaction(s) went to completion within about 1min. Reaction of the blood component(s) with reagents 108 produce colored product 109 which was then detected (Figure 2E), as described below in Example 4.

EXAMPLE 2

Glycated Hemoglobin Assay - Fluidics Structure

A microsystems platform provided by the invention and specifically designed for performing a glycated hemoglobin assay is illustrated in Figure 11.

Construction of the disk embodiments of the platforms of the invention are as described in Example 1. The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber **801**, metering capillary **802**, overflow capillary **803**, metered blood fluid chamber **804** and overflow

5   chamber **805**. As in Example 1, each of the overflow and fluid chambers is also connected with air ports or air channels, such as **814**, and capillary junction(s) **815**, that permit venting of air displaced by fluid movement on the platform.

Blood fluid chamber **804** was fluidly connected to capillary **810** that was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm and was connected to sacrificial valve **811**.

10   Sacrificial valve **811** was further fluidly connected with capillary **812** that was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm. Capillary **812** was further fluidly connected to mixing chamber **815** that was about 0.25cmcm deep, had a cross-sectional diameter of about 1cm, and was positioned about 2.5cm from the center of rotation. Lysis buffer was loaded directly onto mixing chamber **815** in this embodiment and did not use capillary **818** or lysis buffer chamber **816**

15   as shown in the Figure. 45µL of lysis buffer was applied to the mixing chamber as a solution of 0.1% Triton X100 in 50mM Tris pH 9.5.

Mixing chamber **815** was fluidly connected to capillary **817** that was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm, and was connected to secondary metering structure **819**. Secondary metering structure **819** was about 0.1cm deep and was positioned about 3.5cm from

20   the center of rotation. Secondary metering structure **819** was constructed to comprise two sections. A metering section was arranged proximal to the entry position of capillary **817** and was separated from an overflow section by a septum that extended from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produced a fluid connection between the first metering section having a volumetric capacity of about 6.4µL and the overflow

25   section having an excess volumetric capacity of 90µL. The volumetric capacity of the overflow section was sufficient to accommodate the largest blood fluid volume applied to the disk.

Capillary **821** was in fluid connection with secondary metering structure **819** at the distal wall of the metering section. Capillary **821** was about 0.15cm deep and had a cross-sectional

diameter of about 0.05cm and was connected to boronate affinity matrix chamber **822**. Boronate affinity matrix chamber **822** was about 0.15cm deep, had a cross-sectional diameter of about 0.3cm and was positioned about 4.8cm from the axis of rotation. Boronate affinity matrix chamber **822** was filled with boronate-functionalized agarose beads having a mean diameter of about 60μm; the beads

5   were maintained in the chamber **822** using a porous frit **827**. Fluid flow through capillary 821 was connected to capillary or sacrificial valve **823**. Boronate affinity matrix chamber **822** was further comprised of a translucent window that permitted reflective spectrophotometry of the contents of the chamber.

Boronate affinity matrix chamber **822** was further fluidly connected to capillary **828**.

10   Capillary **828** was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm and was connected to column wash buffer reservoir **829**. Column wash buffer reservoir **829** was about 0.25cm deep and had a cross-sectional diameter of about 2cm and was positioned about 3.6cm from the axis of rotation, more proximal to the axis of rotation than boronate affinity matrix chamber **822**. Column wash buffer reservoir **829** comprises 290μL of column preparation buffer that was a solution

15   of magnesium chloride, taurine, D,L-methionine, sodium hydroxide, antibiotics and stabilizers constituted according to the manufacturer's instructions (Isolab Inc. #SG-6220). Fluid flow through capillary **828** was connected to capillary or sacrificial valve **836**.

Boronate affinity matrix chamber **822** was further fluidly connected to capillary **832**. Capillary **832** was about 0.5m deep and had a cross-sectional diameter of about 0.5mm and was

20   connected to non-glycated hemoglobin read chamber **834**. Chamber **834** was about 0.25cm deep and had a cross-sectional diameter of about 2cm and was positioned about 5cm from the axis of rotation and was further comprised of a translucent window that permitted reflective spectrophotometry of the contents of the chamber at 430nm.

25   As illustrated in Figure 11, in the use of this platform about a 6.4 μL volume of blood was applied to blood fluid chamber **804**, either directly or using the metering components of the platform described above. Blood flowing through capillary **810** and lysis buffer contained in mixing chamber **815** were mixed in the mixing chamber. A 45μL volume of lysis buffer was mixed with the blood

sample. Fluid flow within mixing chamber **815** was turbulent, in contrast to fluid flow through capillaries **810** or **818,** which was primarily laminar, so that mixing occurred predominantly in mixing chamber **815.** Fluid flow proceeded through channel **817** and into secondary metering structure **819.** The mixture of lysis buffer and blood, comprising a lysed blood sample **841,** flowed

5   at a rotational speed f_7 of about 750rpm into secondary metering structure **819** with release of a sacrificial valve **853.** Lysed blood sample **841** entered and filled the metering section of secondary metering structure **819.** Any additional lysed blood sample then emptied into the overflow chamber of secondary metering structure **819** and filled the total hemoglobin read chamber. Most preferably, a sufficient volume of lysis buffer and blood sample was applied to the disc to fill at least the

10   metering sections of secondary metering structure **819** and the total hemoglobin read chamber.

After the lysed blood sample **841** was completely transferred to secondary metering structure **819,** capillary or sacrificial valve **823** was released, allowing the metered lysed blood sample from the metering section of secondary metering structure **819** through capillary **821** and into boronate affinity matrix **822.** Capillary or sacrificial valve **836** was then released, allowing about 290µL of

15   column wash buffer **843** to flow at rotational speed f_7 through capillary **830** and into boronate affinity matrix **822.** Continued or discontinuous rotation motivates column preparation buffer through boronate affinity matrix **822** and into non-glycated hemoglobin read chamber 834. The control sample read cuvettes 8- and 8~ were then then illuminated by light at a wavelength of 430nm and the blank reading, i.e. reflectance from cuvettes containing only buffer, was determined. The

20   non-glycated hemoglobin read window was then illuminated by light at a wavelength of 430nm and the concentration of non-glycated hemoglobin in the sample that has eluted from the column was determined by reflectance spectroscopy. The total hemoglobin read window was also illuminated by light at a wavelength of 430nm and the concentration of total hemoglobin in the lysed sample was determined by reflectance spectroscopy.

25   The amount of non-glycated hemoglobin in the sample is determined by dividing the amount of hemoglobin obtained by illuminating the eluted fraction by the amount of hemoglobin obtained in the total fraction.

EXAMPLE 3

Combination Glucose Concentration - Glycated Hemoglobin Assay Platform

A microsystems platform provided by the invention and specifically designed for performing

5   both a determination of blood glucose concentration and a glycated hemoglobin assay is illustrated in Figures 12A through 12Q and 13A through 13E.

Construction of the disk embodiments of the platforms of the invention were as described above. Figure 13B shows a detailed description of the microfluidics components of the platform, which are described in additional detail below. Figure 13C shows the geometry of a screen printed

10   electrical lead layer deposited on a mylar substrate. Figure 13D shows the positions of screen printed heaters activated by the electrical leads of the lead layer and screen printed on mylar. Figure 13E shows a overlay of these components in the assembled disc.

Referring to the microfluidics components of the platform shown in Figure 13B, an entry port 1 is positioned on the top surface of the disc and is open   for the user to apply an unmetered

15   sample. Entry port 1 is about 0..32cm deep, has a cross-sectional diameter of about 1cm, is positioned about 2cm from the center of rotation, and is fluidly connected to capillary channel 1A that is about '1mm deep and has a cross-sectional diameter of about 1mm. Capillary channel 1A is fluidly connected to metering component 2, which comprises four sections. The first section is rectangularly-shaped and extends in a direction proximal to the axis of rotation away from its fluid

20   connection with capillary channel 1A. About half way up this rectangular section is a lateral chamber 3, which empties into a blood glucose metering chamber 4 and an overflow chamber 5. The first section of the metering component is about 0.1cm deep, has a cross-sectional diameter of about 0.4cm, is positioned about 2.2cm from the center of rotation, and has a volumetric capacity of about 5microliters for Hb assay. The lateral chamber 3 is 1mm deep, has a cross-sectional diameter of

25   1mm, and is positioned 2.3cm from the center of rotation. Blood glucose metering chamber 4 is 1mm deep, has a cross-sectional diameter of 4.5mm, is positioned 2.6cm from the center of rotation and has a volumetric capacity of 16μL. Overflow chamber 5 is 1mm deep, has a cross-sectional

diameter of 2mm, is positioned 2.6cm from the center of rotation and has a volumetric capacity of 7μL.

Overflow chamber 5 is fluidly connected to overflow channel 8 that is 0.75mm deep, has a cross-sectional diameter of 0.75mm and extends 3.8cm from overflow chamber 5. Overflow channel 5 is fluidly connected to short sample detection cuvette 9 that is two depths: 0.25mm and 2.2mm(inner) deep, has a cross-sectional diameter of 2.5mm, is positioned 4-5.8cm from the center of rotation and has a volumetric capacity of 50μL.

Blood glucose metering chamber 4 is fluidly connected to capillary 15 that is about 0.25mm deep, has a cross-sectional diameter of about 0.25mm and extends about 0.1cm from blood glucose metering chamber 4. Capillary 15 is connected to sacrificial wax valve 6, which is further fluidly connected with wax recrystallization chamber 6A that is about 0.5mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary 15 controlled by the valve. Capillary 15 is further fluidly connected with glucose assay chamber 11 that is about 0.1cm deep, has a cross-sectional diameter of about 0.5mm, is positioned about 5cm from the center of rotation and has a volumetric capacity of about 10μL. Glucose assay chamber 11 comprises a depression 11A in the surface of the platform having a depth of about 0.1cm, most preferably comprising a circular or rectangular depression connected to capillary 15 so that blood flows into the chamber through the bottom of depression 11A. Depression 11A is constructed to have a volumetric capacity of from half to twice the assay volume. Blood glucose assay chamber 11 also comprises a pad or matrix 10 of a positively-charged nylon matrix having a pore size of about 0.8μm. The upper limit on pore size of matrix 10 is chosen to inhibit or prevent blood cell entry into the matrix. The matrix is positioned in blood glucose assay chamber 11 to be in fluidic contact with depression 11A, more preferably covering depression 11A, and most preferably having a surface area greater than the surface area of depression 11A. The matrix was further impregnated with immobilized reagents 11B which produce a detectable product proportional to the amount or concentration of glucose in a blood sample. Most preferably, the detectable product is a colored product 11C, i.e., a product absorbing light at a detectable, most preferably a visible, wavelength.

Lysis metering chamber **2** is fluidly connected to capillary **7** controlled by sacrificial valve **7A**. Sacrificial wax valve **7A** is further fluidly connected with wax recrystallization chamber **7B** that is 1mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **7** controlled by the valve. Capillary **7** is 0.25mm deep, has a cross-sectional diameter of 0.25mm, extends 0.1cm from lysis metering chamber **2** and is fluidly connected to blood lysis chamber **16**. Blood lysis is contained within the mixing chamber Blood lysis chamber **16** is 2.3mm deep, has a cross-sectional diameter of 1cm, is positioned 3cm from the center of rotation, and contains 45μL of blood lysis solution (0.1% Triton-X100 in 50mM Tris, pH 9.5).

Blood lysis chamber **16** is fluidly connected at a distal aspect to capillary **17** controlled by sacrificial valve **18**. Capillary **17** is about 0.25mm deep and has a cross-sectional diameter of about 0.25mm Sacrificial wax valve **18** is further fluidly connected with wax recrystallization chamber **18A** that is about 0.5mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **17** controlled by the valve. Capillary **17** is fluidly connected to secondary metering structure **19**. Secondary metering structure **19** is 1.5mm deep, and is positioned 3.8cm from the center of rotation. Secondary metering structure **19** is constructed to comprise three sections. A first section **20** comprises a throwaway section that is used to discard the first sample and taking the second sample to provide a better sample for analysis, having a volumetric capacity of about 6.4μL. Throw away section **20** is arranged proximal to the entry position of capillary **17** and is separated from a metering section **21** by a septum that extends from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produces a fluid connection between throwaway section **20** and the metering section **21**. Metering section has a volumetric capacity of about 6.4μL and is fluidly connected to an overflow section **24** having an excess volumetric capacity of about 90μL. The volumetric capacity of the overflow section is sufficient to accommodate the largest blood fluid volume applied to the disk.

Capillary **25** is in fluid connection with secondary metering structure **21** at the distal wall of the metering section. Capillary **25** is about 0.15cm deep and has a cross-sectional diameter of about

0.5mm and is connected to boronate affinity matrix chamber **28**. Capillary **25** is fluidly connected to sacrificial wax valve **26** that is further fluidly connected with wax recrystallization chamber **26A** Wax recrystallization chamber **26A** is about 0.3cm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary

5   **25** controlled by the valve.

Boronate affinity matrix chamber **28** is about 0.15cm deep, has a cross-sectional diameter of about 0.3cm and is positioned about 4.8cm from the axis of rotation. Boronate affinity matrix chamber **28** is filled with boronate-functionalized agarose beads (Isolab, $SG-6220) having a mean diameter of about 60μm; the beads are maintained in the chamber **28** using a porous frits **29**.

10   Boronate affinity matrix chamber **28** is further fluidly connected to capillary **31**. Capillary **31** is about 0.25mm deep and has a cross-sectional diameter of about 0.25mm and is connected to column wash buffer reservoir **30**. Column wash buffer reservoir **30** is about 0.25cm deep and has a cross-sectional diameter of about 2cm and is positioned about 3.6cm from the axis of rotation, more proximal than boronate affinity matrix chamber **28**. Column wash buffer reservoir **30** comprises

15   about 290μL of column wash buffer comprising a solution of asparagine, magnesium chloride, taurine and D, L-methionine (Isolab, #SG-6220). Fluid flow through capillary **31** is connected to sacrificial valve **32**. Sacrificial wax valve **32** is further fluidly connected with wax recrystallization chamber **32A** that is about 0.5mm deep and has a volumetric capacity sufficient to sequester melted wax from a released wax valve and prevent occlusion of the lumen of the capillary **32** controlled by

20   the valve.

Boronate affinity matrix chamber **28** is further fluidly connected to capillary **37**. Capillary **37** is about 0.5mm deep and has a cross-sectional diameter of about 0.5mm and is connected to sample collection cuvette array **12**. Sample collection cuvette array **12** is about 0.25cm deep and has a cross-sectional diameter of about 2.1cm and is positioned about 5cm from the axis of rotation.

25   Sample collection cuvette array **12** is separated into a multiplicity of individual chambers, each separated from one another by septa that extend from the distal wall of the cuvettes to a position adjacent to the proximal wall of the cuvettes, so that a fluid passage **50** is maintained between each of the cuvettes. The fluid passage **50** is formed by the back (proximal wall) of the sample collection

cuvette array **12** and the row of septa separating each of the sections of the sample collection cuvettes **12**. Capillary **33** is fluidly connected to sample collection cuvette array **12** at a position adjacent to the proximal wall of the array and directed to the cuvette most proximal to the boronate affinity matrix chamber **28**.

5   Capillary **22** is fluidly connected to secondary metering structure **19**. Capillary **22** is about 0.25mm deep and has a cross-sectional diameter of about 0.25mm and is connected to secondary metering structure **19** at a position between the metering section and the overflow section. Capillary **22** is further fluidly connected with total hemoglobin read chamber **23**. Total hemoglobin read chamber **23** is about 0.25cm deep, is positioned about 5cm from the center of rotation, and has a

10   volumetric capacity of about 50μL. Total hemoglobin read chamber **23** is positioned radially more distal from the center of rotation than secondary metering structure **19**, and comprises a read window translucent to light having a wavelength of 300-1000nm. In addition, there is no capillary or sacrificial valving controlling fluid flow in capillary **23**.

The platform also comprises control sample read cuvettes **13** and **14**, advantageously

15   positioned in proximity to total hemoglobin read chamber **23**. Control sample read cuvettes **13** and **14** are each about 0.25cm deep, positioned about 5cm from the center of rotation, and have a volumetric capacity of about50μL. Control sample read cuvettes **13** and **14** comprise a read window translucent to light having a wavelength of 410nm. Control sample read cuvettes **13** and **14** are not fluidly connected to any other structure on the platform.

20   Air displacement channels **33** and capillary junction(s) **34**, that permit venting of air displaced by fluid movement on the platform, are fluidly connected to the components of the platform to permit unimpeded fluid flow.

As illustrated in Figures 12 A through 12Q, in the use of this platform a volume of blood about 30μL is applied to entry port **1**. Blood enters lysis subvolume **2** and lateral passageway **3**

25   under the influence of gravity and capillary forces in the absence of rotation of the platform, as shown in Figure 12A. Upon rotation of the platform at a first rotational speed f_1 of 400-600rpm, blood completely fills lysis subvolume **2** and also flows through passageway **3** and into blood glucose metering chamber **4** and overflow chamber **5**, shown in Figure 12B. Blood is retained in

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blood glucose chamber **4** either due to capillary pressure or by a sacrificial valve **6**, most preferably a wax valve. Similarly, blood is retained in blood lysis subvolume **2** by a valve, most preferably a sacrificial valve **7**. Excess blood flows at rotational speed f_1 through overflow channel **8** and into overflow chamber **9**, shown in Figures 12C and 12D. Typical values for the first rotational speed are an acceleration of about 20 to 60 rpm/sec to a final radial velocity of about 600rpm.

After blood is metered and excess blood delivered to overflow chamber **9**, the rotational speed of the disc is reduced to a rotational speed f_{1a} of 600 to 100rpm, typically 60rpm, to perform a blanking measurement on sample collection cuvette array **12**, total hemoglobin read chamber **23** and blood glucose assay chamber **11**. Measurements of blanking cuvettes **13** and **14** are also advantageously performed.

The disc is then accelerated to a second rotational speed f_2 greater than f_1, and typically in the range of 800rpm to about 1000rpm. At this speed, capillary valve **6** is overcome or sacrificial valve **6** is released, and about 16μL of blood from blood glucose metering chamber **4** flows through capillary **15** and into blood glucose assay chamber **11**, shown in Figure 12E. Upon entering assay chamber **11**, blood fluid components are forced into absorbent matrix **10** through depression **11A**. The blood fluid is incubated in matrix **10** for a time sufficient for the reagents **10A** to produce a colored product **10B** in an amount proportional to the amount of glucose in the blood fluid sample. The disc is slowed, typically to a rotational speed f_4 of about 100rpm, for glucose data acquisition using reflectance spectrometry; data acquisition as the disc is spinning down also enables to instrument to set t=0 for the assay, based on a decrease in reflectance when the matrix **10** is wet by the blood fluid components and hence the matrix's scattering decreases. Development of colored product **10B** is shown in Figure 12G.

The disc is then accelerated to rotational speed f_3 of about 1000rpm, with release of sacrificial valve **7A** and fluid flow of about 5 - 6.4μL of blood from metered subvolume **2** through capillary **7** and into blood lysis chamber **16** containing about 40μL of blood lysis buffer. This is shown in Figure 12H. The mixture of blood and blood lysis buffer in blood lysis chamber **16** is mixed by agitation, wherein the platform is accelerated repeatedly +250 rpm/sec to -250 rpm/sec

(wherein "+" and "-" indicate acceleration in different directions), typically 250-500rpm/sec, over a time period of about 2 min, typically 1-3 min, as shown in Figures 12I and 12J.

The disc then is accelerated to a rotational speed f_s of 1000rpm and sacrificial valve **18** is released. Lysed blood from blood lysis chamber **16** flows through capillary **17** and into secondary metering structure **19**, as shown in Figure 12K. The lysed blood solution sequentially fills throwaway section **20**, which is used as a trap for cell debris, metering section **21** and excess lysed blood then fills overflow section **24**. Filling of metering chamber **21** is immediately followed by fluid flow through capillary **22** and filling of total hemoglobin read chamber **23**. The disc is spun at rotational speed f_s for a time sufficient to substantially completely drain blood lysis chamber **16**. The configuration of the blood fluids on the disc after this spin is shown in Figure 12L.

The disc is then decelerated to a rotational speed of 750 rpm, and sacrificial valve **26** is released. A metered volume of about 6.4μL of lysed blood from metering section **21** flows through capillary **27** and into boronate affinity matrix chamber **28** (shown in Figure 12M). The lysed blood solution is allowed to incubate in the chamber for a time of about 1 min, sufficient for glycated hemoglobin to bind to the matrix. This aspect of the disc is illustrated in Figure 12N.

The disc is then accelerated to a rotational speed of 1000rpm, and sacrificial valve **32** is released. A volume of about 290μL of column wash buffer (Isolab #SG-6220) flows from wash buffer reservoir **30** though capillary **31** and into boronate affinity matrix chamber **28** (shown in Figure 12O). The wash buffer displaces the non-glycated hemoglobin and other components of the lysed blood fluid from the affinity column matrix and into sample collection cuvette array **12**. Figures 12P through 12Q show sequential filling of the individual cuvettes in sample collection cuvette array **12**. The rotation speed of the disc is reduced, to 60 rpm for sample collection cuvette array **12** and total hemoglobin read chamber **23** to be interrogated spectrophotometrically. The glycated fraction of the blood sample is determine algorithmically by subtracting the non-glycated hemoglobin fraction in sample collection cuvette array **12** from the total hemoglobin detected in total hemoglobin read chamber **23**.

EXAMPLE 4

Blood Glucose Assay - Chemistries

Microsystems platforms as provided by the invention were used to perform blood glucose and glycated hemoglobin assays as described herein.

Blood Glucose Assay

A series of blood glucose assays were performed to determine the precision of repeated glucose assays using the microsystems platforms of the invention.

The assays were performed on a microsystems platform according to Examine 3 using a round reagent cuvette (113). 60-70μL of human whole blood was applied to the disk, and 15μL metered into the metering capillary. A glucose reagent pad obtained from LifeScan was used for performing the glucose determination. Optical absorbance readings were obtained at 430nm, 590nm and 660nm on the unreacted reagent pad as a control. Blood was released into the reaction chamber and optical data gathered at one-second intervals for about 1 min. Absorbance at 590nm was specific for the amount of glucose in the sample; absorbance at 430nm was specific for hemoglobin in the sample; and absorbance at 660nm was used to detect non-specific background absorbance, as described above.

A total of seven assays were performed for each of two samples and the amount of glucose (in mg/dL) determined as follows:

	Sample 1	Sample 2
Glucose mg/dL	136.0	113.2
%CV	4.4%	4.5%

These assays were repeated as above using a microsystems platform according to Example 1 comprising a rectangular reagent cuvette (113). A Biodyne-B membrane (Pall) was impregnated with glucose oxidase reagents as described above and used for performing the glucose determination. In these embodiments, chamber 113 had dimensions of 4mm x 5mm.

The glucose determination was performed exactly as described in the first series of experiments. Standard blood glucose assays were run on these samples in parallel to compare the results obtained with the microsystems platforms of the invention with conventional assays. These results were as follows:

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mg/dL Reference Method	mg/dL Invention
46.8	61.9
85.9	76.8
107.0	115.8
149.0	144.2

These results established that the microsystems platforms of the invention were capable of determining blood glucose concentrations.

10   Correction for Interfering Substances

In addition, assays were performed on a microsystems platform to Example 3 using a round reagent cuvette (113) and the glucose results obtained were corrected for hemoglobin interferences as discussed herein. In these experiments, the spectrophotometric results obtained were used to calculate blood glucose with and without correction for the hemoglobin absorbance signal at 590nm.

15   The correction factor was determined empirically using samples comprised of phosphate buffered saline to which known qualities of glucose and/or hemoglobin were added. The resulting samples were assayed on the microsystems platform according to Example 3 using a round reagent cuvette (113) to give the following data:

Sample	Glucose Concentration (mg/dL)	Hemoglobin Concentration (mg/dL)	Reflectance(590nm)	Reflectance(430nm)
A	0	0	0.0152	-0.0204

20

B	0	1.25	0.2116	0.3041
C	75	5	1.2767	0.3452
D	78	0	0.9576	0.1311

For glucose chromagen alone, Sample D, the Reflectance(430nm) is 16.1% of the Reflectance(590nm) [(0.131-(-0.0204)/(0.9576-0.152)]. For hemoglobin alone, Sample B, the Reflectance(590nm) is 60.5% of the Reflectance (430) [(0.2116-0.0152)/(0.3041-(-0.0204))]. For the combination of glucose and hemoglobin, Sample C, the Reflectance(430nm) is the sum of the Reflectance (430nm) due to the hemoglobin plus 16.1% of the Reflectance(590nm) due to the glucose chromagen; similarly, the Reflectance(590nm) is the sum of the Reflectance(590nm) due to the glucose chromagen plus 60.5% of the Reflectance(430nm) due to the hemoglobin. Using standard algebraic methods, the factor to be used was calculated to be:

Reflectance(590nm) due to glucose chromagen = Reflectance(590nm) – F x Reflectance(430nm) where F = 0.274

The results shown in Figures 14A and 14B illustrate the difference in the accuracy of the data obtained with and without correcting for the hemoglobin absorbance signal.

Glycated Hemoglobin Assay

A series of glycated hemoglobin assays were performed to determine the precision of repeated binding of glycated hemoglobin by measuring the amount of hemoglobin put on a phenyl boronate column and the amount of (non-glycated) hemoglobin that could be eluted from the column.

These experiments were performed using a microsystems platform of the invention as disclosed in Example 3; the phenyl boronate column material was from a kit obtained from IsoLab Glyc-Affin GHb. 60-70μL of human whole blood was applied to the disk, and 5μL metered into and mixed with 45μL of lysis buffer in the mixing chamber of the platform. 5μL of the lysed blood was metered onto the phenyl boronate column and non-glycated hemoglobin eluted with 290μL of elution buffer. The reflective absorbance of the lysed blood and the eluted non-glycated hemoglobin

was determined spectrophotometrically at 430nm and used to calculate the amount of non-glycated hemoglobin in the sample.

Seven separate assays were performed on each of 2 difference whole blood samples. These results are as follows:

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	Sample 1	Sample 2
%NGHb, reference method	93	90
%NGHb, invention	93	92
%CV	1.2%	1.7%

DNA binding assays

These experiments were performed to show that the microsystems platforms of the invention

10   could efficiently isolate plasmid DNA from a sample. In these experiments, diatomeceous earth was used to specifically bind plasmid DNA, and the results were compared with a standard plasmid DNA extraction kit (Qiagen).

In these experiments, the Qiagen assay was performed until the bacterial culture had been disrupted with chaotropic salts. At this point, the solution was split in half, and one half assayed

15   using the Qiagen reagents and columns, and the other half 300μL assayed using a microsystem platform adapted from the one described in Example 2. In this embodiment, the diatomeceous earth column was loaded onto the platform and the sample applied thereto by centrifugation at about 800rpm. The column was washed twice with 100μL of Qiagen buffer PE at about 850rpm, and the plasmid DNA was then eluted twice, with 50μL of Qiagen Buffer EB, once at 600rpm and again at

20   at 900rpm. The eluted plasmid DNA from each preparation was precipitated by the addition of ice cold ethanol to 70% and the DNA pellet collected by centrifugation and resuspended in 50μL of 10mM Tris-HCl, pH 8. 15μL were analyzed by conventional agarose gel electrophoresis, stained with ethidium bromide and osverved under ultraviolet light illumination.

The results of these assays are shown in Figure 15, and illustrate the use of the microsystems platforms of the invention for isolating plasmid DNA from a biological fluid sample.

It should be understood that the foregoing disclosure emphasizes certain specific
5   embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.